JOURNAL OF DAIRY SCIENCE

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Vol. XXX, No. 9, September, 1947

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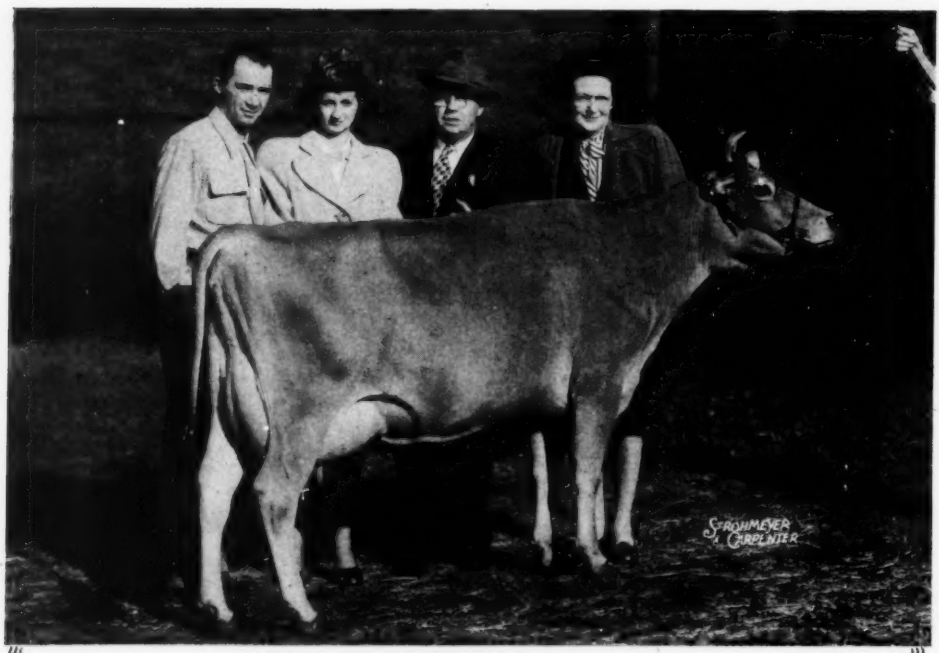

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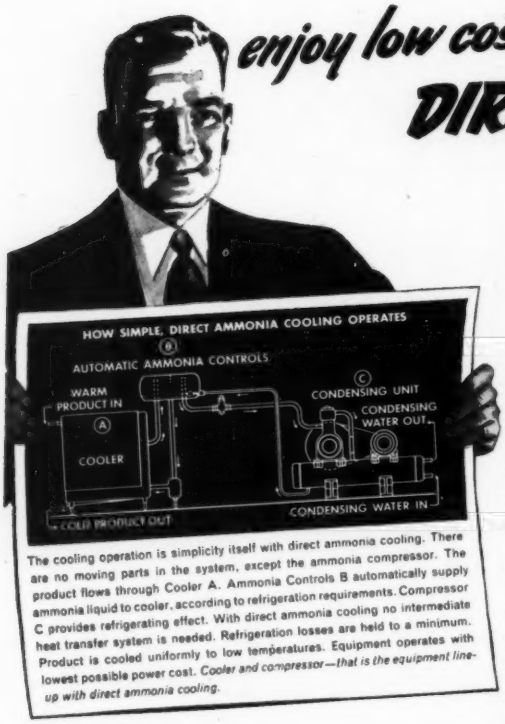

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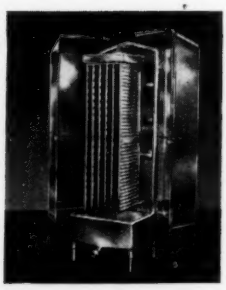

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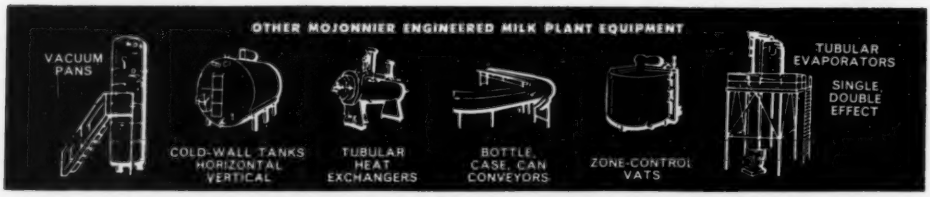

JOURNAL OF DAIRY SCIENCE

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THE RELATION OF SEASON OF FRESHENING TO MILK PRODUCTION

GEORGE E. FRICK, A. I. MANN, AND STEWART JOHNSON

University of Connecticut, Storrs

In an effort to determine the relation of season of freshening to milk production, a study was made of 22,212 Dairy Herd Improvement Association lactation records. These records were obtained in Connecticut during the years 1924 through 1945, although the majority of them were obtained in the later years of this period.

EXPERIMENTAL

The amount of milk produced by cows freshening in each month had been computed by the University Dairy Department for the 305 days following the date of freshening. In many instances the cows were dry before this period of time had elapsed and in such cases the production covered a shorter milking period. Data for each breed were tabulated separately. The breed distribution was 9,325 Guernseys, 8,260 Holsteins, 2,344 Ayrshires, and 2,283 Jerseys. The lactation records were sorted not only by breed but also by the age of the cows at time of freshening. Two-year-olds accounted for 22 per cent of the total number of records, 3-year-olds for 21 per cent, and 4-year-olds for 16 per cent. Milk yields were used as reported and were not adjusted to a common butterfat test.[1]

Received for publication April 18, 1947.

[1] As milk is priced in Connecticut and in many fluid milk markets of the East, the amount of milk produced is much more important than the amount of butterfat. For this reason the lactation records were not adjusted to give yields of 4 per cent fat-corrected milk. In April, 1947, for example, the price paid Connecticut wholesale producers for 3.7 per cent milk averaged $5.53 per cwt., and the butterfat differential was six cents per point. An increase in butterfat test from 3.7 to 4.0 per cent would bring a price premium of only 18 cents per cwt. A corresponding increase in the volume of milk (8.1 per cent) would bring a price premium of 45 cents per cwt.; an increase in the volume of milk equivalent to the increased energy content of 4.0 per cent milk as contrasted to 3.7 per cent milk (about 5.3 per cent for Holstein cows) would bring a price premium of 29 cents per cwt. Moreover, if the records had been converted to a common butterfat base, the conclusions of the study would not have been modified significantly. For example, yields for Holstein cows in the most favorable month would have exceeded those in the least favorable month by 13.9 per cent, rather than by 14.9 per cent as noted in the analysis of records of actual volumes.

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TABLE 1

Average milk yields, by ages, for 9,325 Guernsey cows freshening in different months

Month of freshening	Age at date of freshening, in years													No. of cases
	1	2	3	4	5	6	7	8	9	10	11	12 and over	All ages*	
	(lb. milk per cow, 305-day lactation period)													
Jan.	5790	6700	7080	7580	8000	8590	8330	8430	8680	8800	8030	8070	7540	825
Feb.	6020	6680	7390	7900	8100	8210	8160	8440	8340	8630	8370	7780	7610	723
March	5550	6660	7210	7520	7870	8270	8640	8240	8530	8530	7960	8520	7500	776
April	5140	6530	6980	7650	7850	8150	8030	8130	7970	7870	9100	8210	7360	712
May	4500	6330	6850	7750	7900	7780	7780	7970	7760	8300	7000	7200	7230	626
June	5160	6110	6640	7220	7070	7220	7330	7380	7210	6970	6850	6200	6800	659
July	5930	6110	6530	6950	6820	7260	7140	7130	6890	6800	5650	6340	6670	783
Aug.	4760	6140	6810	7250	7320	7370	7750	7360	7440	7500	8960	.....	6950	786
Sept.	6120	6130	6750	7230	7520	7500	7310	7710	7410	7410	6300	7250	6950	859
Oct.	6070	6620	7080	7500	7970	7840	8240	7950	8050	8720	7040	8100	7380	928
Nov.	6300	6630	7340	7860	8070	7960	8260	8170	8650	6920	7450	6950	7520	828
Dec.	5560	6610	7110	7940	8190	8510	8690	7740	7520	8760	8460	6840	7550	820
All months†	5580	6440	6980	7530	7720	7890	7970	7890	7870	7930	7600	7410	7260	.....
No. of cases	98	2196	1950	1479	1194	880	612	422	235	133	67	59	9325	9325

* These averages were obtained by weighting the yield for each age group by the total number of cows in that age group regardless of month of freshening.

† These averages were obtained by dividing the 12-month total of the average monthly yields by 12.

RESULTS

Month of calving and milk yields, by breeds. As shown in table 1, of the nine age groups of Guernseys with more than 100 cases, January was the most favorable month in four instances, December in three, and February in two. July was the least favorable in seven of these groups, June in one, and June and July yields were equal in one. The term "favorable" as used here is based on the volume of milk produced by cows freshening in each month. It is not based on the value of these yields, which would be influenced by the seasonal pattern of milk prices, or on the yields relative to costs of production.

For all Guernsey cows, yields were highest for those freshening in February and lowest for those freshening in July. Average production for cows calving in February was 7,610 lb. and for cows calving in July was

TABLE 2

Relative importance of various months of calving for Guernsey cows in different age groups

Age at date of freshening, in years	Percentage of total cows freshening during year that calved in:											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1 & 2	9	7	6	7	5	6	7	10	11	11	11	10
3	9	7	8	7	7	6	8	9	10	11	9	8
4	9	8	9	8	6	8	9	7	9	9	10	9
5	9	8	10	9	8	7	8	7	8	11	8	8
6	8	8	10	9	8	7	9	8	7	10	7	9
7	9	9	9	8	8	9	10	7	6	9	8	8
8 & over	10	9	10	9	6	9	8	7	8	8	7	8
All ages	9	8	8	8	7	7	8	8	9	10	9	9

6,670 lb. The difference in favor of cows freshening in February was 940 lb. From February until July average yields decreased for cows freshening in each succeeding month, and from July until February average yields increased from each month to the next.

The average yield for all cows freshening in a given month was calculated by weighting the yield for each age group by the total number of cows in that age group regardless of month of freshening. This method was chosen because of the joint relationship of age with season of freshening. A larger-than-usual number of 1- and 2-year-old Guernseys freshened in the fall months but this tendency gradually disappeared as the cows became older (table 2). A simple average, with total production for all cows freshening in a given month being divided by the number of cows, would be influenced by the proportion of cows which were of a young age. If the weighting factor in each age group is the total number of observations for the year, possible errors due to the joint relationship of season of freshening with age are avoided. If weighted averages according to the method described had not been used, February through July yields would have erred on the high

TABLE 3

Average milk yields, by ages, for 8,260 Holstein cows freshening in different months

Month of freshening	Age at date of freshening, in years													No. of cases
	1	2	3	4	5	6	7	8	9	10	11	12 and over	All ages*	
	(lb. milk per cow, 305-day lactation period)													
Jan.	6870	7930	9470	10270	10510	10920	10340	10370	10460	11290	11180	10170	9720	744
Feb.	5600	8480	9830	10520	10900	11040	10990	11780	11330	11140	8320	10920	10120	685
March	7370	8260	9190	10330	10610	11080	10680	11110	9780	11330	9490	9650	9790	691
April	7290	8200	9620	10030	10640	10560	10710	10860	10670	10440	9800	8800	9770	568
May	5880	8010	8520	10120	10100	10740	10930	9710	10490	9680	9830	9120	9390	487
June	7000	8120	8840	9610	9560	9880	9920	10200	9830	9280	9230	11140	9200	549
July	7690	7930	8740	8970	9560	9670	9990	9360	8980	9770	8770	8390	8930	722
Aug.	7330	7740	8120	9460	9450	9360	9650	9210	10000	9880	9240	9320	8810	796
Sept.	8150	8090	9630	9420	9810	10040	10860	9760	9830	10200	9500	9170	9220	777
Oct.	6500	8020	9010	9960	10050	10690	10890	10500	10990	10920	9870	9450	9540	760
Nov.	7400	8440	8900	10060	10580	10340	10690	10380	10750	10650	12400	8750	9650	749
Dec.	8080	8750	9080	9990	10270	10270	10480	9890	11500	10390	11280	11420	9700	732
All months†	7100	8160	9000	9900	10170	10380	10510	10260	10380	10410	9910	9690	9490	.....
No. of cases	81	1698	1739	1336	1094	787	548	393	249	163	84	88	8260	8260

* These averages were obtained by weighting the yield for each age group by the total number of cows in that age group regardless of month of freshening.

† These averages were obtained by dividing the 12-month total of the average monthly yields by 12.

side, and August through January yields would have erred on the low side; the extent of error would have been as large as 80 lb. in August and September and 110 lb. in March.

Average yields of Holsteins were highest for cows freshening in February and lowest for cows freshening in August (table 3). The most favorable month was the same as for Guernseys, but the least favorable was a month later in the summer. Milk yields were 14.9 per cent greater for cows freshening in the most favorable month than in the least favorable month, slightly greater than the corresponding difference of 14.1 per cent noted with Guernsey cows.

Thirty-three per cent of the 1- and 2-year-olds freshened in the September–November quarter, but this fall-freshening tendency gradually disappeared as age increased, with only 21 per cent of the cows 8 years old and over freshening in this quarter (table 4). This corresponds closely with the association of age with season of freshening as found with Guernsey cows.

TABLE 4

Relative importance of various months of calving for Holstein cows in different age groups

Age at date of freshening, in years	Percentage of total cows freshening during year that calved in:											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1 & 2	9	8	6	5	4	6	9	10	12	11	10	10
3	10	8	8	6	6	5	8	11	11	10	10	8
4	8	8	10	7	6	7	8	10	10	8	10	9
5	10	9	8	7	7	7	9	8	7	9	9	10
6	10	9	9	7	6	7	10	9	9	8	8	9
7	8	9	11	10	5	7	9	9	5	8	8	11
8 & over	7	8	10	10	9	9	10	8	6	8	7	7
All ages	9	8	8	7	6	7	9	10	9	9	9	9

Milk yields were highest for Ayrshire cows freshening in January and lowest for those freshening in July (table 5). Yields in the most favorable month exceeded those in the least favorable by 16.2 per cent.

As with the Ayrshires, milk yields were highest for Jersey cows freshening in January and lowest for those freshening in July (table 5). The difference in yields between the most favorable and least favorable months was greater than for any of the other three breeds; cows freshening in January produced 19.2 per cent more than those freshening in July.

Tests of significance of averages. Although there was considerable dispersion about the average milk yield for cows freshening in each month, enough observations were made so that the differences noted were highly significant. Standard errors of the difference between means were used in judging reliability. Comparing the average yield for Guernseys freshening in February with that for Guernseys freshening in July, the actual difference was about ten times the standard error of the difference between the

means. This was very highly significant. Between two consecutive months differences were sometimes small and were not always significant. In general, however, average yields consistently increased from the least favorable to the most favorable month, and consistently decreased from the most favorable to the least favorable month.

Month of calving and milk yield—all breeds combined. The general patterns of the association of month of calving with milk yields were similar for each of the four breeds. Similarly, the general patterns were similar for each of the most important age groups, although the differences in yields between the most favorable and least favorable month of calving became relatively greater as age increased. These differences were sufficiently small,

TABLE 5

Average milk yields, by breeds and for all breeds combined, for 22,212 cows freshening in different months

Month of freshening	Average milk yields*					No. of cases
	Guernseys	Holsteins	Ayrshires	Jerseys	All breeds combined	
	(lb. milk per cow, 305-day lactation period)					
Jan.	7540	9720	8520	7460	8450	1946
Feb.	7610	10120	8240	7230	8570	1766
March	7500	9790	8170	7250	8400	1874
April	7360	9770	7880	6890	8260	1630
May	7230	9390	7750	6420	8000	1492
June	6800	9200	7790	6570	7770	1572
July	6670	8930	7330	6260	7540	1876
Aug.	6950	8810	7620	6340	7650	2002
Sept.	6950	9220	7890	6630	7860	2046
Oct.	7380	9540	8070	6900	8210	2114
Nov.	7520	9650	8260	7010	8340	1990
Dec.	7550	9700	8280	6940	8360	1904
Av.	7260	9490	7980	6820	8120	22212

* Yields for each breed were calculated as described in tables 1 and 3. Yields for all breeds combined were calculated by weighting yields for each breed by the total number of observations, *i.e.*, 9,325 Guernseys, 8,260 Holsteins, 2,344 Ayrshires, and 2,283 Jerseys.

however, so that a single measure of the relationship of month of calving to milk yields was considered to be of value.

Average yields were highest for cows calving in February and lowest for cows calving in July (table 5). The difference in yields was 1,030 lb. Cows freshening in February produced 13.7 per cent more milk than those freshening in July. Yields increased from one month to the next from July to February, and decreased from one month to the next from February to July.[2]

[2] In applying the relationships found in this analysis to individual farms, it must be kept in mind that they are for the average cow in dairy herd improvement associations in Connecticut. Feeding rates, pasture conditions, milk-producing capacity of cows, etc., might vary from the D.H.I.A. situation on any particular farm, and thus affect the relationship of month of calving to milk yields.

DISCUSSION

Woodward (2) studied the influence of the month of calving on milk production of 15,442 cows in dairy herd improvement associations in 12 states. The lactation records covered a 365-day period, rather than the 305-day period for the records used in the Connecticut analysis. Milk yields were corrected to a 4-per cent milk-equivalent basis. This was not done in the Connecticut study, since the price differential for butterfat places primary importance on milk yield rather than butterfat test.

A comparison of the association of month of calving with milk yields as found in the two studies is shown in figure 1. Milk yields for cows calving

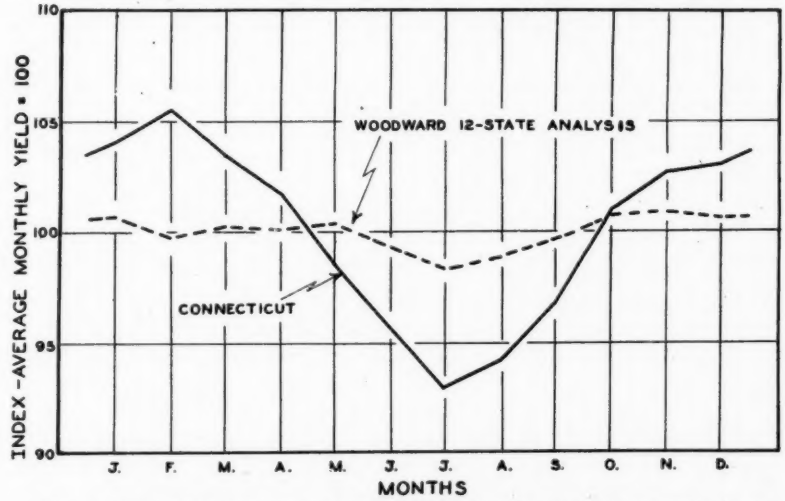

FIG. 1. Milk yields for cows calving in different months, 22,212 Connecticut lactation records compared with 15,442 12-state records analyzed by Woodward.

in the most favorable month in Woodward's study were only 2.5 per cent higher than for cows calving in the least favorable month. In the Connecticut analysis, milk yields in the most favorable month exceeded those in the least favorable month by 13.7 per cent. The range between yields in the low and high months was more than four times greater with Connecticut records than with the 12-state records used by Woodward. In absolute terms, milk yields in the most favorable month exceeded those in the least favorable month by 222 lb. in Woodward's study and by 1,030 lb. in the Connecticut study. The relatively small influence of month of calving on milk yields found by Woodward may have been due to his combining all records—all breeds, all ages, all states—before calculating average yields. If climatic differences should cause the association of month of calving with milk yields to vary among the different states, a combination of records from

several states would tend to minimize fluctuations in yields. Greater variability might have been noted in Woodward's analysis if the effect of the joint relationship of age with season of freshening had been eliminated.

Although in both studies the month of July was found to be the least favorable month of calving, the most favorable month in the Connecticut study came in February rather than in November, as in Woodward's study. The reasons for this difference are not apparent.

In a recent New Hampshire study, Morrow *et al.* (1) reported average milk yields by breeds for 4,030 cows calving in different months. Produc-

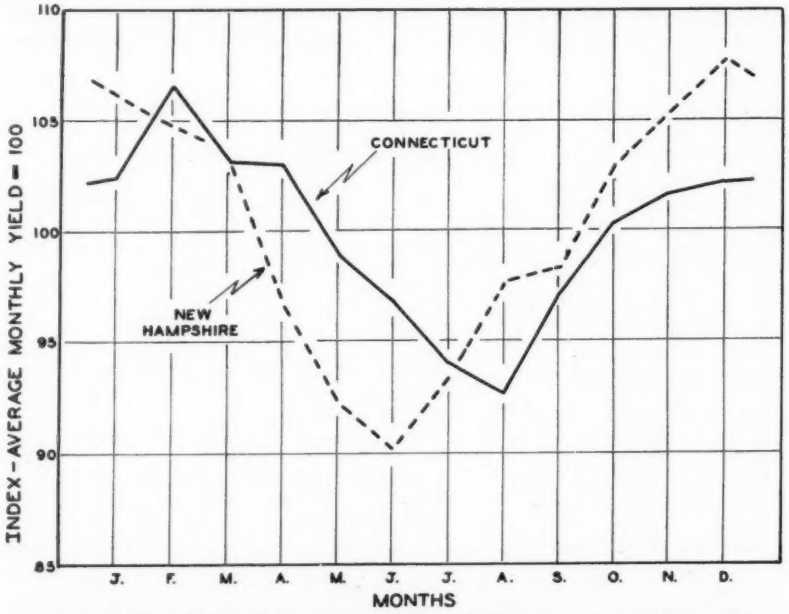

FIG. 2. Milk yields for Holstein cows calving in different months, 1,946 New Hampshire lactation records compared with 8,260 Connecticut records.

tion was calculated on the basis of a 305-day lactation period, and corrected to a 4-per cent milk-equivalent, mature-equivalent volume.

As shown in figure 2, the most favorable month of freshening for Holstein cows, according to the New Hampshire analysis, was December, and the least favorable was June. Average milk yields for cows freshening in December exceeded those freshening in June by 19.2 per cent. This variation was greater than that found in Connecticut, where the corresponding difference was 14.9 per cent. The most favorable month in Connecticut was February, and the least favorable month was August.

As shown in figure 3, Guernsey cows calving in January gave the most

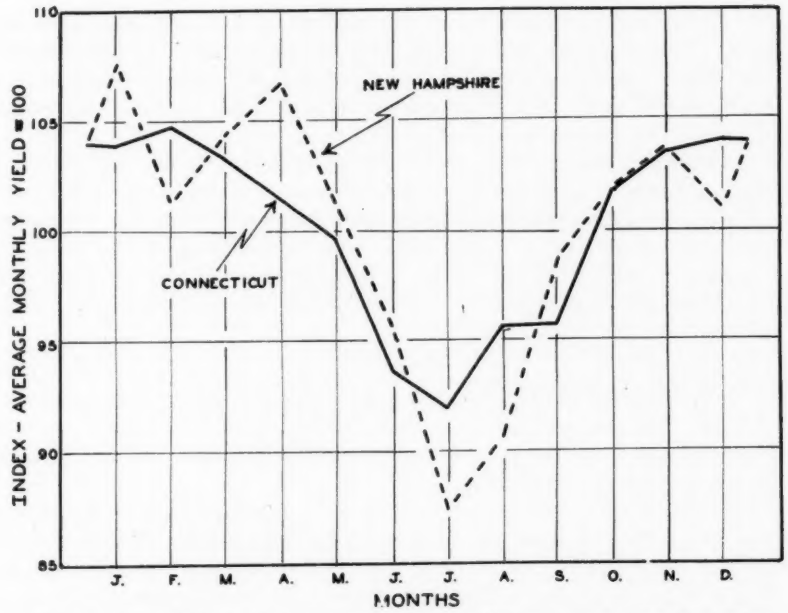

FIG. 3. Milk yields for Guernsey cows calving in different months, 747 New Hampshire lactation records compared with 9,325 Connecticut records.

TABLE 6

*Relation of month of calving to milk yield, D.H.I.A. herds in New York State, 1936–1945**

Month of freshening	Lb. milk produced per cow										
	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	10-yr. average
Jan.	8519	8247	8423	8410	8636	8830	9302	9482	9436	9598	8888
Feb.	7897	8047	8150	8032	8248	8606	8939	9245	9045	8895	8510
March	7752	7862	8078	7980	8265	8239	9141	9138	8736	8888	8408
April	7676	7786	7614	7781	8027	8108	8467	8603	8743	8661	8147
May	7721	7542	7666	7463	7847	8122	8473	8690	8430	8679	8063
June	7852	7952	7427	7465	7877	7712	8442	8500	8654	7999	7988
July	8085	7475	7838	7389	7950	8028	8334	8681	8292	8506	8058
Aug.	8542	8617	8059	7910	8047	8490	8695	8891	8614	8810	8468
Sept.	8590	8217	8341	8418	8434	8889	9177	9103	9262	9223	8765
Oct.	8687	8901	8833	8502	8429	8972	9391	9684	9380	9532	9031
Nov.	8676	8797	8958	8492	8768	9247	9383	9926	9471	9552	9127
Dec.	8116	8573	8432	8344	8680	9045	9309	9538	9199	9475	8871

* Annual data obtained from J. F. Kendrick, Bureau of Dairy Industry, Washington, D. C., and from W. T. Crandall, Department of Animal Husbandry, Cornell University, Ithaca, New York. The 1936–1945 averages were computed by the authors of this report.

milk, according to the New Hampshire analysis, and those in July gave the least. The low month was the same as in the Connecticut analysis, but the high month was 1 month earlier. Production in the most favorable month exceeded that in the least favorable month by 23.1 per cent in New Hampshire and 14.1 per cent in Connecticut.

A summary based on records of Dairy Herd Improvement Associations in New York State indicated that cows freshening in November gave the most milk and cows freshening in June gave the least milk (table 6). The least favorable month came 1 month earlier than in Connecticut, and the most favorable month 3 months earlier. The difference in yield between the most favorable and the least favorable month was about the same as in Connecticut.

SUMMARY

Milk yields for 22,212 Connecticut cows averaged highest for cows freshening in February and lowest for cows freshening in July. The most favorable month of calving came later in the fall-winter season than had been found in studies in other areas.

Production for cows calving in the most favorable month of freshening exceeded that in the least favorable month by 13.7 per cent. This was much greater than found by Woodward in a 12-state analysis but less than was found in recent studies in New Hampshire, and about the same as found in New York.

The association of milk yield with month of calving was similar for each of the four breeds—Guernsey, Holstein, Ayrshire, and Jersey. The association also was similar for each of the most important age groups, although the difference in yields between the most favorable and least favorable month of calving became relatively greater as age increased.

In determining differences in yields associated with month of calving, statistical procedures taking into account the joint relationship of age with season of freshening were necessary. As the cows in Connecticut herds increased in age, a smaller proportion freshened in the fall months of the year.

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CHOLINE STUDIES WITH YOUNG DAIRY CALVES. II. EFFECT OF MILK AND OTHER SUPPLEMENTS ON BLOOD CHOLINE LEVELS[1]

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In a previous publication (11) the authors presented evidence which indicates that the choline content of colostrum is reflected in the choline content of the blood of the young calf. The following study is a report of a continuation of this work. The experiments were designed to show trends of choline levels of blood with increasing age, and to determine effects of milk and other supplements in the diet upon the blood choline levels of young calves.

EXPERIMENTAL

In these experiments the method of analysis for choline in blood was the same as that used previously (11).

Effect of removal of milk from the diet. Trial I included seven calves. These calves were kept in individual pens and allowed the amount of grain mixture they would clean up twice daily. The ingredients of this mixture are shown in table 1. Alfalfa hay was fed *ad libitum* after the first 2 weeks of age. Two calves received milk throughout the trial. For the other five calves the removal of milk was started at 4 weeks of age and was completed by the fifth week.

Figure 1 shows the results of this trial. Blood choline values definitely were depressed by the removal of milk from the calves' diets. This decrease was obvious even by the end of the fifth week, when the removal of milk was complete.

Trial II was conducted to obtain more evidence of the blood choline decline incident with removal of milk and was designed similar to trial I. However, a different grain mixture was used, as shown in table 1. This mixture contained about 1 mg. of choline per gram. Vitamins A and D were fed by capsule to insure a daily intake of 10,000 I.U. of vitamin A and 400 units of vitamin D for the first week, after which the amounts were decreased 50 per cent. All the dams of these calves had been on pasture just prior to calving, and the milk given these calves was from Holstein cows receiving some pasture. The Holstein calves received 10 lb. of milk daily and the Jersey and Guernsey calves, 8 lb. Four calves were used as controls and received milk throughout the trial. Milk was removed from the diets of

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[1] Journal Paper no. 301 of the Purdue University Agricultural Experiment Station.

[2] Now at North Carolina State College of Agriculture, Raleigh.

TABLE 1

Composition of grain mixtures

	Trial I	Trial II
	(*lb.*)	(*lb.*)
Ground yellow corn	32	25
Rolled oats	25	28
Wheat bran	15	15
Linseed oil meal	10	10
Soybean oil	.....	20
Commercial casein	10	.....
Alfalfa leaf meal	5	.....
Iodized salt	1	1
Steamed bone meal	.....	1
Mineral mixture*	1	.....
Cod liver oil	1	.....

* Bone meal, 50%; limestone, 46.7%; ferric citrate, 3%; copper sulphate, 0.1%; manganese, 0.1%; and cobalt chloride, 0.1%.

11 calves at the fifth week of age. The results of this trial are shown in figure 2. The differences in blood choline levels of the two groups were not as great as were observed in trial I but were found to be statistically significant. If trial II had been terminated at 7 weeks, the results of the two trials would have been very similar. The calves were not as thrifty as those in trial I. The incidence of scours was greater and appeared to cause a definite lowering of blood choline. Calves which became unthrifty at one time or another, as evidenced by low weight gains or loss in weight, usually had low or fluctuating blood choline values. The comparison of the two groups,

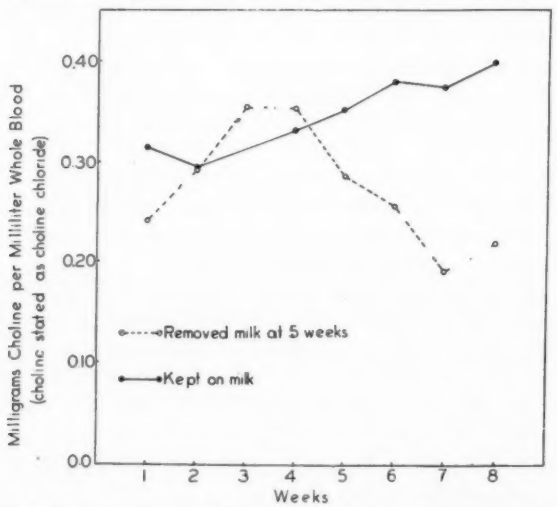

FIG. 1. The effect of milk consumption on the choline content of the blood of calves in trial I.

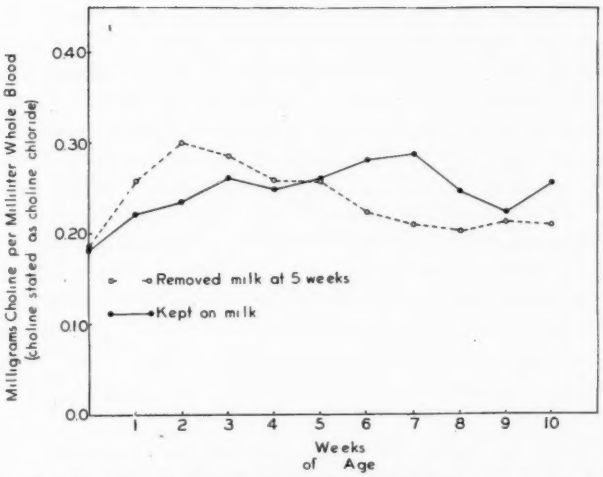

FIG. 2. The effect of milk consumption on the choline content of the blood of calves in trial II.

shown in figure 2, indicates that removal of milk from the calves' diets caused a lowering of blood choline.

Effect of feeding choline and methionine on blood choline. The removal of milk from the diet caused lower blood choline levels. Whether this depression of blood choline was due either to insufficient choline or to insufficient labile methyl groups (7, 8, 9, 10) was tested by feeding choline and methionine. Four calves which had not received milk after 5 weeks of age were used in this trial. For a period of 2 weeks, beginning at the eighth week of age, each calf was given by capsule a daily dosage of 12 g. of choline chloride, followed for 2 weeks by a daily dosage of 4 g. of choline chloride and 6 g. of dl-methionine.

The data of table 2 indicate that these supplements had no effect upon the

TABLE 2

Effect of feeding choline and methionine on blood choline levels (mg. choline chloride per ml. whole blood)

	Age in weeks	JP317*	B587†	P124*	B588†	Av.
Initial	8	0.23	0.26	0.24	0.22	0.24
12 g. choline chloride daily	9	0.30	0.21	0.19	0.23	0.23
	10	0.24	0.16	0.25	0.21	0.22
4 g. choline chloride and 6 g. methionine daily	11	0.27	0.22	0.24	0.15	0.22
	12	0.26	0.22	0.22	0.19	0.22

* Jersey.
† Holstein.

level of blood choline. The weekly variations of blood choline values were not statistically significant. An insufficient quantity of choline or of labile methyl groups does not appear to be responsible for lowered blood values which occurred after the removal of milk from the diet.

Effect on blood choline of returning milk to the diet. A test was made to determine the effectiveness of the addition of milk to diets with and without choline supplements in raising blood choline values. Milk was restored to the diets of the same four calves which had received the choline and methionine supplements. The milk was increased gradually over a period of 4 days, after which the Jersey calves received 8 lb. of milk a day and the Holsteins, 10 lb. In addition to the milk, two of these calves were given 8 g. of choline chloride daily. As controls, four other calves of the same age received no milk. This trial began when the calves were 13 weeks of age and continued for 4 weeks. The results are given in table 3. The data on these small num-

TABLE 3

Effect of restoring milk to the diet on the choline content of blood (mg. choline chloride per ml. whole blood)

Weeks	Control—no milk or choline					Milk		Milk & choline		Av.
	P125*	80B†	B589†	B582‡	Av.	JP317*	P124*	B587†	B588†	
Initial	0.20	0.24	0.27	0.24	0.24	0.26	0.22	0.22	0.19	0.22
1	0.24	0.22	0.26	0.21	0.23	0.30	0.28	0.25	0.23	0.27
2	0.26	0.27	0.26	0.26	0.26	0.29	0.28	0.29	0.28	0.29
3	0.22	0.21	0.22	0.19	0.21	0.30	0.35	0.25	0.22	0.28
4	0.23	0.15	0.22	0.22	0.21	0.31	0.28	0.31	0.30	0.30

* Jersey.
† Holstein.
‡ Guernsey.

bers should not be considered conclusive but it appears that the milk (and milk and choline) caused an elevation of blood choline when introduced into the diet of these calves which had received no milk for 7 weeks. The supplementation of the milk with choline chloride appeared to be without effect.

Attempts to increase the blood choline by injections of choline chloride. Since oral administration of choline chloride did not appear to increase the choline content of the blood, possibly either the choline was not absorbed or its accumulation in the blood stream was prevented by rapid removal. In the previous experiments, the blood samples were taken approximately 24 hours after giving the choline. Therefore, even if the intestinal absorption of the choline were rapid and the subsequent removal from the blood were rather slow, any initial rise in the choline content of the blood following feeding the choline chloride might not have been detected. However, with the introduction of choline chloride directly into the blood stream, it was thought that an elevation of the choline level should be detected unless the removal from the blood is extremely rapid.

Choline chloride solutions were injected into one jugular vein, and blood samples were taken from the opposite jugular for choline analysis. The injected solution contained 1 g. of choline chloride per 4 ml. of water, and injections were made at the rate of about 1 g. of choline chloride per minute. The injection of 1 g. of choline chloride into an 80-lb. calf should be sufficient to approximately double the choline content of the blood.

The blood choline values obtained just before and at intervals following the injections are shown in table 4. The first injection was made into calf no. 79B, a Holstein heifer. This calf lacked thrift and had made slow weight gains, but an autopsy at a later date showed no pathological symptoms. This calf became prostrate within 60 seconds after the injection and showed difficulty of breathing, considerable salivation, some belching of gas, and defeca-

TABLE 4

Effect of injecting choline chloride on blood choline levels (mg. choline chloride per ml. whole blood)

Calf	80B*	79B*	79B	79B	B591†	B591	B591
Date (1946)	Nov. 11	Nov. 4	Nov. 11	Nov. 19	Nov. 11	Dec. 2	Dec. 17
G. choline injected	0	1	1	2	1	1	2
Calf weights (lb.)	165	80	82	84	80	97	107
Before	0.24	0.23	0.14	0.16	0.22	0.24	0.14
3 min.‡	0.25	.....	0.24	0.50	0.25	0.22	0.30
6 "	.....	0.28	0.23	.....	0.21	0.16	0.16
10 "	0.24	.....	.....	0.32	.....	.....	Dead
15 "	0.20	0.18	0.13	Dead	0.20	0.18	.....
30 "	0.21	.....	0.17	.....	0.21	0.17	.....
60 "	0.23	0.20	0.18	.....	0.17	0.15	.....
240 "	0.25	0.20	.....	.....	0.22	0.20	.....

* Holstein.
† Guernsey.
‡ Minutes following beginning of injection.

tion and urination within the first 3 minutes. When the calf was helped to its feet after 5 minutes, it was very unstable, with considerable quivering of the muscles. The calf appeared normal 15 minutes after injection. These reactions were typical of all injections except that they did not always cause complete collapse.

The increases following 1 g.-injections were not consistent. The 2 g.-injections produced a definite increase in the choline of the blood although this amount proved fatal. It appears that the injected choline was removed quickly from the blood stream. To check the possibility that handling and repeated bleeding might influence the choline levels, calf no. 80B was bled at intervals without injection. These effects were insignificant.

DISCUSSION

It might appear that there is a simple correlation between the choline intake with milk and the choline level of the blood. As shown in previous work (11), the choline content of the blood increased considerably when the

calves received colostrum, which is high in choline. This response might be analogous to the response of vitamin A in the calves' blood due to the high vitamin A content of colostrum. In this study the choline content of blood remained high until the withdrawal of milk, when it decreased. The choline content also appeared to be elevated by returning milk to the diet.

That these responses are not directly related to the choline of the diet is indicated by two observations. In the first place, when the milk was removed from the diets, the calves consumed about 1 lb. more grain per day. The choline content of this additional grain (0.45 g.) largely compensated for the loss of choline due to the removal of milk (0.63 g.). In the second place, administration of additional choline and methionine with the grain ration failed to increase blood choline levels. Consequently, the drop in blood choline hardly can be attributed to the lack of choline or transferable methyl groups, since the dietary intake of choline cannot be correlated with the blood choline levels.

Since there does not appear to be a simple quantitative relation between choline intake and blood levels, it might appear that the choline of the grain mixture was less available than that in the milk. Some work with poultry (5) indicates that the choline of some grain mixtures is not utilized as well as in others. The supplementation of the grain mixture with comparatively large amounts of choline chloride would be expected to cause increases in blood choline, even though utilization of the added choline was very inefficient.

Inasmuch as milk favored higher choline levels than grain, possibly milk contains choline precursors. Studies with chickens indicate that poultry are capable of some choline synthesis and that some feeds contain choline precursors (4, 6). However, the supplementation of the grain with choline chloride and methionine should have compensated for any precursors of choline of the milk diet.

Inasmuch as choline is present as lecithin in both grains and milk, this would not explain the differences. However, Artom and Cornatzer (1) recently observed that in rats more synthesis of phospholipids occurred in the intestinal walls when fat and choline were ingested than when choline or fat alone was ingested. Fishman and Artom (3) also have shown that liver lecithin is greater when rats are fed high fat diets than when fed low fat diets, if adequate choline is present. The calves, while receiving milk, did have considerably more fat in their diets than when milk was removed. It may be that the greater amount of fat necessitated more phospholipid formation for efficient utilization of the fat, and the higher amounts of choline occurred in the blood because of the type of nutrient being transported. On the basis of these observations (1, 3), it seems plausible that the differences in fat content of the two diets might be related to differences found in blood choline levels.

In consideration of the evidence previously presented, it appears that some component of milk other than choline or methionine may influence blood choline levels of young calves.

Choline chloride is toxic when injected intravenously. This is in considerable contrast to the apparently low toxicity when fed orally. No deleterious effects were noted when as much as 12 g. of choline chloride per day was given orally, but it may be that prolonged feeding of these rather large amounts would produce undesirable results. The injected choline chloride was removed from the blood stream very rapidly. The lethal amount of choline chloride for 80- to 100-lb. calves when injected at a rate of about 1 g. per minute appeared to be between 1 and 2 g.

SUMMARY

1. Removal of milk from the diets of calves at 5 weeks of age resulted in a drop in blood choline values.

2. Feeding choline chloride or choline chloride and methionine did not increase blood choline levels.

3. While not conclusive, some data are presented that indicate returning milk to the diet of calves caused elevation of blood choline.

4. Evidence is presented which indicates that a component of milk other than choline or methionine may influence blood choline levels.

5. Intravenous injections of choline chloride produced only a temporary rise of the choline content of the blood. It was rapidly removed from the blood stream. Injections of 2 g. proved to be fatal.

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THE PHARMACODYNAMICS OF ASCORBIC ACID IN CATTLE. I. ABSORPTION AND ELIMINATION[1, 2]

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It has been believed generally that cattle synthesize sufficient quantities of ascorbic acid to meet their requirements. Several investigators (20, 30, 33, 34) fed dairy cattle for extended periods on diets low in ascorbic acid without observing significant decreases in blood plasma ascorbic acid or decreased excretion of the vitamin in milk or urine. Others (9, 21, 22, 32) have reported that oral administration of crystalline ascorbic acid in large quantities did not increase the ascorbic acid content of the milk. Whitnah and Riddell (35) and Riddell *et al.* (22) fed 65 kg. of green rye to dairy cows which were otherwise kept under barn feeding conditions. They observed several-fold increase in the urinary excretion of ascorbic acid and a doubling of the blood plasma levels during the first 12 hours. The blood plasma and urinary ascorbic acid returned to pre-feeding levels within 24 hours. No significant changes occurred in the milk ascorbic acid as a result of feeding green rye.

Rasmussen *et al.* (19) and Knight *et al.* (9) have shown that intravenous or subcutaneous administration of crystalline ascorbic acid results in significant increases in this compound in cows' milk. Investigations of rumen contents by Whitnah and Riddell (35), Knight *et al.* (10), and Vavich *et al.* (32), both *in vivo* and *in vitro*, have shown that ascorbic acid is lost rapidly. Crystalline ascorbic acid was destroyed more rapidly than that from plant sources, probably because of the slower release from the plant tissues; Vavich *et al.* (32) showed that ascorbic acid destruction was decreased but not entirely prevented in autoclaved rumen contents.

The probable synthesis of ascorbic acid by the dairy cow has been suggested (8, 10, 11, 20, 22, 31, 33, 34), but the site of synthesis is unknown.

It generally is believed that blood plasma ascorbic acid is increased most effectively when vitamin C is administered subcutaneously or intravenously. Hawley *et al.* (7), Knight *et al.* (10), and Satterfield *et al.* (24) have revealed that blood levels rise very rapidly following intravenous administration, with excretion in the urine practically being completed within 2 hours.

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Subcutaneous administration results in a slower rise in blood levels, with a correspondingly slower rate of excretion. Oral administration of chlorobutanol (2, 3, 14, 15, 16, 17, 26) significantly increases blood plasma ascorbic acid in cattle and urinary excretion of ascorbic acid is increased greatly (16, 17). This phenomenon also has been demonstrated in rats (12, 28). Feng (6) reported that chlorobutanol and phenobarbitol accelerate the urinary excretion of ascorbic acid by rats and increase its concentration in small intestinal tissue, kidney, spleen, and liver.

Although the bovine apparently does not require extraneous ascorbic acid in the diet, considerable quantities of the vitamin are ingested, especially when animals are on good pasture. In view of the controversial nature of ascorbic acid utilization and its indicated destruction in the rumen, the study of absorption and elimination of ascorbic acid when administered into various known levels of the digestive tract, as compared to subcutaneous and intravenous administration, seemed highly desirable. Since chlorobutanol previously had been shown to increase blood plasma ascorbic acid, it seemed advisable to study its effects.

EXPERIMENTAL

Steers were selected for these studies because of the ease of continuous urine collection and the absence of such disturbing side effects as estrus and lactation. Five yearling Hereford cattle averaging 600 lb. at the beginning of the experiment were used. They received daily 6 lb. of a grain mixture composed of four parts shelled corn, two parts whole oats, and one part soybean meal and were fed good quality alfalfa hay *ad libitum*. Feeding and watering were done between 7:00 and 8:00 a.m. and 5:30 and 6:00 p.m. The steers were kept in metabolism stalls for several days before and following each experimental trial and in a small dry lot at other times. Rumen fistulae were made in two steers using the technique of Schalk and Amadon (25), and fistulae were created in the jejunum, cecum, and colon, respectively, in the other three animals, as described by Nichols (18). Twenty-four-hour urine samples were collected during the experimental trials on all steers except the steer with the small intestinal fistula. The abdominal location of this fistula made urine collection impractical. Ascorbic acid determinations were made on whole blood, blood plasma, urine, feces, and ingesta, using the method of Roe and Keuther (23). Hemoglobin was determined by the method of Evelyn (5) and blood cell volume by the method of Wintrobe (36). All colorimetric determinations were made with the Evelyn photoelectric colorimeter.

Twenty-four-hour urine samples were collected before trials, during trials, and 1 to 2 days afterwards. The urine was collected in 5-l. bottles charged uniformly with 100 c.c. of 20 per cent metaphosphoric acid. Urine specimens were measured and sampled uniformly between 7:30 and 8:00

a.m. and 5 : 30 and 6 : 00 p.m. All statistical analyses were made according to Snedecor (27).

RESULTS

Control. Since preliminary trials indicated that blood plasma ascorbic acid was decreased by frequent bleeding, this factor was investigated. Two trials on each of four steers (those with rumen, colon, and cecum fistulae) were made. Base levels of urinary excretion of ascorbic acid and blood content of the vitamin were established. The animals then were injected with 50 c.c. of distilled water into their various fistulae and were bled ten times during an 8-hour period. The results, as shown in table 1, reveal that whole blood and blood plasma ascorbic acid levels were significantly decreased, as compared to base levels (mean of −24, 0, and 24 hours used uniformly in this and succeeding trials discussed in this section), during the period when bleeding times were close together. These levels returned to pre-trial levels between the fourth and sixth hours. The extreme drop in blood plasma ascorbic acid for the fourth hour is not readily explainable, but in six of the eight trials this value was lower than for the third hour and in seven trials it was lower than for the sixth hour. The urine excretion for this period (table 2) shows little variation for the 3-day periods involved. The variance between steers, however, was highly significant.

Rumen. Six trials were made on each of two steers. Sixteen grams of ascorbic acid, dissolved in 100 c.c. of distilled water, were delivered onto the floor of the ventral rumen sac with a syringe and catheter. This procedure was repeated every other day for three trials; the steers then were given a 13-day rest period before the second series of three trials was run. As shown in table 1, significant increases in whole blood and blood plasma ascorbic acid were observed 15 and 30 minutes after placing the vitamin in the rumen. Whole blood ascorbic acid was increased over base levels for the first 4 post-injection hours, but blood plasma values dropped noticeably after 1.5 hours.

Inspection of the data for individual steers reveals that in eleven of twelve trials both whole blood and blood plasma ascorbic acid were increased; the maximum response occurred 15 minutes after injection in ten trials. In some trials increased levels were maintained for 4 hours after treatment, but variation in the duration of response and the tendency for repeated bleeding to lower blood ascorbic acid made average increases over base levels after 30 minutes rather small. In table 2 the urine response is recorded for each steer and for both rumen steers. The steers with rumen fistulae showed approximately the same increase in urinary elimination on the day of treatment as compared to the day before and the day after. The variance between days and between steers for the two rumen steers analyzed together was significant ($P < 0.05$), while the variance between trials × steers was non-significant.

TABLE 1

Effects of administration of ascorbic acid into the digestive tract on blood ascorbic acid

Administration of ascorbic acid		No. of sample series	Interval of bleeding[a]	Ascorbic acid			
				Whole blood		Blood plasma	
					Diff.[b]		Diff.[b]
(*g.*)	(*site*)			(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)
0	Control[c] series	8	−24 hr.	0.418	.....	0.270	.....
			0	0.410	.....	0.282	.....
			15 min.	0.395	−0.021	0.316	0.027
			30 min.	0.391	−0.025	0.300	0.011
			1 hr.	0.352	−0.064[d]	0.269	−0.020
			1½ hr.	0.340	−0.076[d]	0.250	−0.039
			2 hr.	0.386	−0.030	0.295	0.006
			3 hr.	0.395	−0.021	0.294	0.005
			4 hr.	0.372	−0.044[e]	0.200	−0.089[d]
			6 hr.	0.446	0.030	0.356	0.067[e]
			8 hr.	0.445	0.029	0.301	0.012
			24 hr.	0.419	.....	0.315	.....
16	Floor ventral rumen sac	12	−24 hr.	0.568	.....	0.474	.....
			0	0.603	.....	0.492	.....
			15 min.	0.698	0.110[d]	0.598	0.113[d]
			30 min.	0.667	0.079[d]	0.548	0.063[e]
			1 hr.	0.634	0.046	0.517	0.032
			1½ hr.	0.625	0.037	0.528	0.043
			2 hr.	0.638	0.050	0.467	−0.018
			3 hr.	0.614	0.026	0.503	0.018
			4 hr.	0.639	0.051[e]	0.478	−0.007
			6 hr.	0.587	−0.001	0.469	−0.016
			24 hr.	0.592	.....	0.488	.....
4	Cecum	10	−24 hr.	0.455	.....	0.370	.....
			0	0.519	.....	0.389	.....
			15 min.	0.624	0.133[d]	0.527	0.147[d]
			30 min.	0.561	0.070	0.479	0.099[d]
			1 hr.	0.587	0.096[e]	0.447	0.067[e]
			1½ hr.	0.555	0.064	0.439	0.059
			2 hr.	0.570	0.079	0.431	0.051
			3 hr.	0.556	0.065	0.459	0.079[e]
			4 hr.	0.528	0.037	0.426	0.046
			6 hr.	0.508	0.017	0.390	0.010
			24 hr.	0.498	.....	0.381	.....
4	Colon	10	−24 hr.	0.457	.....	0.362	.....
			0	0.525	.....	0.410	.....
			15 min.	0.596	0.104[d]	0.491	0.115[d]
			30 min.	0.548	0.056	0.449	0.039
			1 hr.	0.575	0.083[e]	0.446	0.036
			1½ hr.	0.591	0.099[e]	0.478	0.068
			2 hr.	0.601	0.109[d]	0.449	0.039
			3 hr.	0.536	0.044	0.439	0.029
			4 hr.	0.500	0.008	0.340	−0.036
			6 hr.	0.481	−0.011	0.370	−0.006
			24 hr.	0.494	.....	0.356	.....

TABLE 1 (*Continued*)

Administration of ascorbic acid		No. of sample series	Interval of bleeding[a]	Ascorbic acid			
				Whole blood		Blood plasma	
					Diff.[b]		Diff.[b]
(*g.*)	(*site*)			(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)
Summary	Rumen, cecum, colon	32	−24 hr.	0.498	.....	0.407	.....
			0	0.552	.....	0.434	.....
			15 min.	0.643	0.116[d]	0.540	0.122[d]
			30 min.	0.597	0.070[d]	0.495	0.077[d]
			1 hr.	0.601	0.074[d]	0.473	0.055[d]
			1½ hr.	0.591	0.064[d]	0.485	0.067[d]
			2 hr.	0.605	0.078[d]	0.450	0.032[e]
			3 hr.	0.571	0.044[e]	0.469	0.049[d]
			4 hr.	0.561	0.034	0.419	0.001
			6 hr.	0.529	0.002	0.413	−0.005
			24 hr.	0.532	.....	0.413	.....
4	Small intestine (jejunum)	8	−24 hr.	0.319	.....	0.176	.....
			0	0.328	.....	0.226	.....
			15 min.	0.442	0.115[e]	0.355	0.150[d]
			30 min.	0.525	0.198[d]	0.388	0.183[d]
			1 hr.	0.426	0.099[e]	0.360	0.155[d]
			1½ hr.	0.366	0.039	0.276	0.071
			2 hr.	0.372	0.045	0.290	0.085
			3 hr.	0.415	0.088[e]	0.288	0.083
			4 hr.	0.356	0.029	0.269	0.064
			6 hr.	0.389	0.062	0.309	0.104[e]
			24 hr.	0.335	.....	0.214	.....

[a] F-ratios between intervals were highly significant for each type of experiment reported below.

[b] Interval means compared to mean of −24, 0, and 24 hr.

[c] Distilled water injected into rumen, cecum and colon—4 steers, 2 trials each.

[d] Highly significant mean difference.

[e] Significant mean difference.

TABLE 2

Effects of administration of ascorbic acid into the digestive tract on urinary ascorbic acid

Administration of ascorbic acid		No. of 24-hr. samples in each period	Av. daily excretion of ascorbic acid in urine		
			Day before	Day of treatment	Day after
(*g.*)	(*site*)		(*mg.*)	(*mg.*)	(*mg.*)
0	Control	8	34.3	40.9	36.5
16	Rumen—1	6	90.6	123.5	75.1
16	Rumen—2	6	58.8	89.0	56.8
Rumen summary		12	74.7	106.3	65.9
4	Cecum	10	46.0	47.4	42.4
4	Colon	10	65.8	72.0	57.6
Rumen, cecum, and colon summary[a, b]		32	63.0	77.2	56.0

[a] Least significant mean difference between days is ±9.6 mg. of ascorbic acid; ±12.8 mg. required for a highly significant mean difference.

[b] Variance between days, between trials, and between steers was highly significant.

Cecum. Ten trials were made on one steer following the same general procedure previously described. Four grams of ascorbic acid in 25 c.c. of distilled water were administered into the cecum with a stomach tube. The average maximum increase in blood ascorbic acid occurred 15 minutes after treatment (table 1); both whole blood and plasma showed substantial increases for the first 3 hours, with a gradual decline to base levels thereafter. In some trials the maximum response occurred as late as 3 hours. Variation in the exact site of administration probably is responsible for the variation in time of maximum increase. Urinary excretion of ascorbic acid (table 2) was not significantly increased during the 24-hour period after administration of ascorbic acid, and the between-trial variance was not significant.

Colon. Four grams of ascorbic acid were administered in 25 c.c. of distilled water during each of ten trials on one steer. One-half of the ascorbic acid solution was injected approximately 18 inches anterior to the fistula and the remainder approximately 14 inches from the opening. As shown in table 1, maximum response in blood ascorbic acid again occurred within 15 minutes after the administration of the vitamin. Whole blood ascorbic acid levels were significantly higher for the first 2 hours, while blood plasma values dropped noticeably after 15 minutes but remained above base levels for the first 3 hours. Positive responses occurred in eleven of twelve trials for whole blood and in ten of twelve trials for blood plasma. The maximum response was delayed in three trials but occurred within the first 2 hours in every case where responses were noted. As shown in table 2, a slight increase in urinary excretion of ascorbic acid was observed for the day of treatment, but the variance between days and between trials was non-significant.

Rumen, cecum, and colon summary. Since administration of ascorbic acid into various levels of the digestive tract revealed approximately the same type of response in blood ascorbic acid, these data were analyzed collectively to increase the total number of replications to 32. The results, as shown in table 1, indicate that increases in blood ascorbic acid could not be detected 4 hours after administration of the vitamin. The observed effects may have been due to the level of ascorbic acid dosage. While four times more ascorbic acid was used in the rumen studies, the amount appearing in the blood at any one time was no greater than when 4 g. were used in the cecum and colon studies. This does not necessarily indicate there was only one-fourth the absorption, since significant increases were observed in urinary elimination of ascorbic acid in the case of the rumen studies, while only slight increases were observed in the colon and cecum studies. A second point to consider is the base level of ascorbic acid in whole blood and blood plasma. The base levels of the four animals throughout these studies frequently were higher than one normally would expect, thus making it less likely that more ascorbic acid would be detected in venous blood over a very long period of time after ascorbic acid was administered.

The urinary excretion of ascorbic acid also was analyzed collectively (table 2). The increased number of replications used in the analysis of variance reveals that highly significant differences occurred between days, between steers, and between trials × steers.

Small intestine. Eight trials were made on a steer with a fistula in the jejunal area of the small intestine. The same bleeding routine was practiced, but no urine specimens could be collected because of leakage from the intestine, which frequently ran down the sheath. Four grams of ascorbic acid in 25 c.c. of distilled water were administered with a stomach tube approximately 24 inches into the intestine. A catheter was fitted with a rubber thumb stall and the fistula opening closed immediately after the ascorbic acid was injected. This device was left in place for the first 90 minutes. This steer later died from intussusception of the intestine at the site of the fistula.

As shown in table 1, maximum response in blood ascorbic acid occurred 30 minutes after administration and persisted at a high level for the first hour. This steer showed a much lower base level of ascorbic acid than the other steers in these experiments. This may account for the 15-minute delay in average maximum response as compared to the other steers. In only two trials out of eight was the maximum blood response noted at 15 minutes, but the other six maxima occurred within the first 90 minutes. The degree of response also was quite variable. In two trials the blood levels were more than doubled while in two others the response hardly was detectable.

Further rumen absorption studies. Since adding ascorbic acid directly onto the rumen floor gave positive evidence of absorption, it seemed desirable to administer ascorbic acid into the intact rumen. The two steers with fistulae in the cecum and colon were used for this study. Sixteen grams of ascorbic acid in 100 c.c. of distilled water were administered for 3 successive days by stomach tube. Blood samples were drawn at 0, 15, and 30 minutes, and at 1, 2, 4, 5, and 24 hours for each trial, and urine samples were collected. The average whole blood and blood plasma ascorbic acid values were not significantly increased (fig. 1) over the base levels (average of 0 and 24 hours used as the base level). A definite positive response did occur in three of the six trials, but this response did not occur regularly at any one time interval. The steers eliminated on the average 82.2 mg. of ascorbic acid the day before and the day after treatment and 110.4 mg. daily during treatment. The magnitude of the increase was essentially the same as in previous trials with rumen fistulae. The variance between days was significant with the first day of treatment, showing increased urine excretion considerably above the day before, and 1 and 2 days after treatment. However, the number of trials is too small to say positively that this observed difference was real. Sixteen grams of ascorbic acid would be

diluted considerably by the rumen contents and it would be difficult to select a bleeding interval that would show if and when maximum absorption occurred.

The administration of 16 g. of ascorbic acid 6 inches into the reticulo-omasal-abomasal orifice also was performed in four trials on the two steers

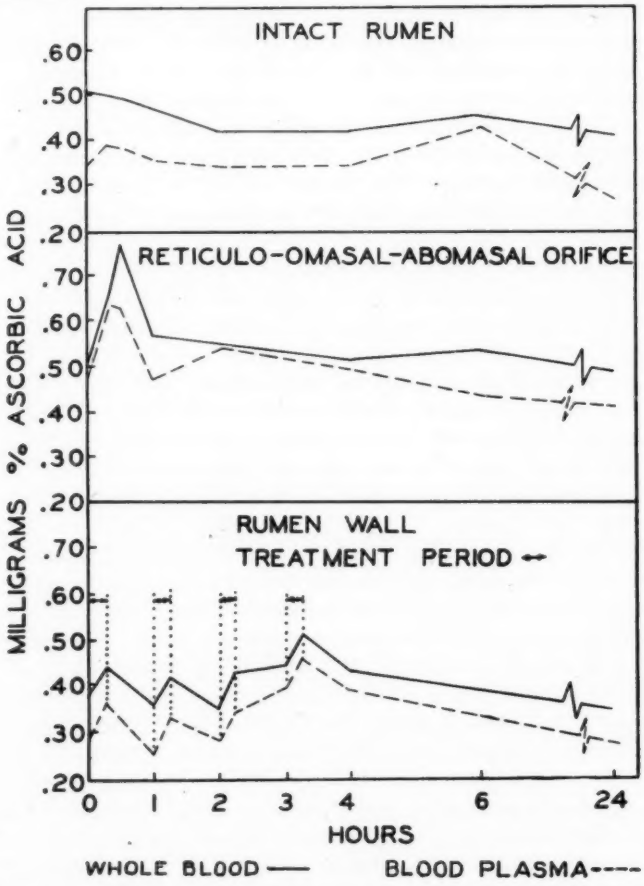

FIG. 1. The effect of administering ascorbic acid in various regions of the rumen on blood ascorbic acid.

with rumen fistulae. Base levels for blood and urine were established and the vitamin given on 2 successive days. The reticulo-omasal-abomasal orifice was located manually and a stomach tube inserted until air blown through it could not be felt bubbling back. Cold ascorbic acid solution

TABLE 3

Effects of administration of ascorbic acid directly into the reticulo-omasal-abomasal orifice

Steer	Urinary ascorbic acid				
	2 days before	1 day before	1st day treatment	2nd day treatment	Day after
	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)
Rumen—1	126.4	156.4	311.0	589.9	183.4
Rumen—2	96.0	23.7	214.2	209.5	137.9
Av.	111.2	90.0	262.6	399.7	160.6

then was passed through the tube. In none of the trials could the solution be felt oozing back into the rumen at the time of administration. The results (fig. 1) reveal that definite absorption occurred from this area. Whole blood ascorbic acid values reached a maximum in the first 30 minutes, while blood plasma values were highest at 15 minutes but remained at a high level through 30 minutes. These mean differences were highly significant. All of the trials showed a marked increase at either 15 or 30 minutes after administration for both whole blood and blood plasma ascorbic acid. Urinary elimination of ascorbic acid, as shown in table 3, was more than doubled the first day of treatment and tripled the second day. The response still was observable on the day following treatment.

Since placing ascorbic acid in an area where it by-passed the rumen gave such a marked positive response of absorption, it seemed highly desirable to see if ascorbic acid is absorbed when held in direct contact with the rumen wall. A petri dish was fitted with a piece of cellulose sponge to serve as the receptacle for ascorbic acid solution. This petri dish and sponge held 80 c.c. of solution and could be held in an upright position without loss of liquid. Six grams of ascorbic acid in 0.5 per cent sodium chloride solution were used for each of 24 trials on two steers. The procedure was to draw a blood sample, hold the solution against the upper ventral rumen wall for 15 minutes, and draw a second blood sample. This

TABLE 4

Holding ascorbic acid solution against the upper ventral rumen wall

Steer	Urinary elimination of ascorbic acid			
	1 day before	1st day treatment	2nd day treatment	Day after
	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)
Rumen—1	183.4	219.4	.....	141.7
Rumen—1	120.4	143.1	115.7	36.4
Rumen—2	137.9	132.8	.....	71.3
Rumen—2	90.5	82.7	42.6	27.4
Av.	133.0	144.5	79.2	69.2

procedure was repeated each hour on each steer for a total of 4 hours on 3 different days. The results, as shown in figure 1, reveal that ascorbic acid increased in both whole blood and plasma after 15 minutes. These differences were highly significant after 3.25 hours as compared to base levels (mean of −24, 0, and 24 hours compared to other means). Urinary elimination, as shown in table 4, was not changed significantly.

Subcutaneous administration. For comparative purposes ascorbic acid was administered subcutaneously to the same four steers on which urine data from previous trials were available. Two grams of ascorbic acid were injected in the foreflank region of each steer and blood samples were drawn at 1, 2, 3, 4, 6, 8, and 24 hours post-injection. A total of eight trials were run. As shown in figure 2, peak levels of ascorbic acid in blood were reached

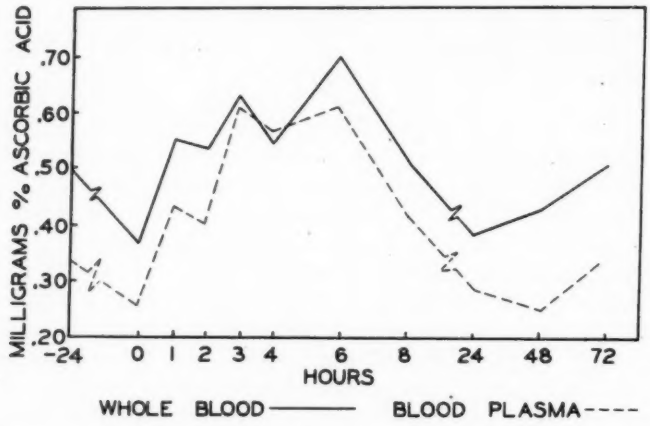

FIG. 2. The effect of subcutaneous ascorbic acid administration on blood ascorbic acid.

within 3 hours after subcutaneous administration and returned to pre-treatment levels within 24 hours. However, all time interval means from 1 to 8 hours after administration were significantly higher than the base levels (mean of −24, 0, and 24 hours afterwards). It should be noted that blood plasma ascorbic acid was higher than whole blood at the fourth hour, with the two being essentially the same at the third hour. Data on individual trials show that maximum blood ascorbic acid values occurred consistently between 3 and 6 hours after administration and blood plasma values were higher than whole blood in six of the eight trials at the fourth hour.

The amount of subcutaneously administered ascorbic acid recovered in the urine was surprisingly small. As shown in table 5, urinary excretion of ascorbic acid increased nearly six-fold but only 9.3 per cent of the administered vitamin was recovered as such in the urine. The range of recovery

TABLE 5

Ascorbic acid recovered in the urine following subcutaneous administration

Steer	No. of samples	Urinary ascorbic acid					Recovered in urine[a]
		2 days before	1 day before	Day of treatment	1 day after	2 days after	
		(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	(%)
Colon	2	89.4	74.2	322.4	59.8	77.9	12.0
Cecum	2	34.7	29.2	183.2	70.2	86.2	7.6
Rumen—1	2	40.8	36.4	191.3	42.0	56.0	6.0
Rumen—2	2	32.8	23.4	131.2	58.2	33.8	5.2
Av.	.....	49.4	40.8	232.0	58.8	63.5	9.3

[a] Mean of day of treatment—mean of 1 and 2 days before/2000 × 100; 2 g. of ascorbic acid were administered subcutaneously.

of ascorbic acid in individual trials varied from 1.5 to 32.5 per cent; both the highest and lowest recovery were observed on the same steer (colon).

Intravenous administration of ascorbic acid. To study speed of removal of ascorbic acid from the blood stream, 2 g. of ascorbic acid were given intravenously during eight trials on four steers. Blood samples were drawn 24 hours before, at 0, 1, 2, 4, 8, 16, 32, 60, and 90 minutes, and at 2, 3, 4, and 24 hours after intravenous administration of ascorbic acid. Urine samples were taken the day before and at 4, 12, 24, and 48 hours after treatment. The results are shown in tables 6 and 7. Intravenously administered ascorbic acid largely disappeared from the blood stream within 30 minutes, with small quantities being demonstrated in the blood for 4 hours.

TABLE 6

Rate of removal of intravenously administered ascorbic acid

No. of sample series	Time of bleeding	Av. ascorbic acid			
		Whole blood		Blood plasma	
			Diff.[a]		Diff.[a]
		(*mg.*%)	(*mg.*%)	(*mg.*%)	(*mg.*%)
8	−24 hr.	0.426	.....	0.234	.....
	0	0.485	.....	0.372	.....
	1 min.	5.05	4.60	6.11	5.82
	2 min.	3.94	3.49	4.60	4.31
	4 min.	2.86	2.41	3.42	3.13
	8 min.	2.20	1.75	2.40	2.11
	16 min.	1.22	0.77	1.54	1.25
	32 min.	0.749	0.299	0.782	0.496
	60 min.	0.538	0.088	0.529	0.243
	90 min.	0.516	0.066	0.460	0.174
	2 hr.	0.532	0.082	0.478	0.192
	3 hr.	0.546	0.096	0.452	0.166
	4 hr.	0.516	0.056[b]	0.469	0.183
	24 hr.	0.440	.....	0.251	.....

[a] Mean of −24, 0, and 24 hr. after.
[b] Not significantly different from base level.

Blood plasma ascorbic acid was considerably higher than whole blood ascorbic acid for the first 16 minutes and remained very near whole blood for the first hour. As shown in table 7, only 12.2 per cent of the ascorbic acid was recovered in the urine. The range of recovery in individual trials was 7.0 to 18.3 per cent. During the day of treatment 53 per cent of the ascorbic acid was eliminated in 4 hours, 33 per cent in the next 8 hours, and 14 per cent in the next 12 hours. This latter figure does not represent much more ascorbic acid than would have been eliminated in 12 hours if no ascorbic acid had been given.

Digestive tract administration of chlorobutanol. Base ascorbic acid levels on the four animals used in the intravenous study were established. Five grams of chlorobutanol then were given daily to each steer for 15 days.

TABLE 7

Ascorbic acid recovery in the urine following intravenous administration

Steer	No. of samples	Av. ascorbic acid in urine						
		Day before	Day of treatment	Day after	Recovery[a]	Day of treatment		
						1st 4 hr.	Next 8 hr.	Next 12 hr.
		(*mg.*)	(*mg.*)	(*mg.*)	(%)	(*mg.*)	(*mg.*)	(*mg.*)
Colon	2	61.8	317.4	86.6	12.8	130.6	110.8	75.9
Cecum	2	86.5	271.5	40.2	9.2	195.2	59.6	16.6
Rumen—1	2	73.0	389.7	53.4	15.8	190.2	136.6	63.0
Rumen—2	2	27.4	251.8	30.2	11.2	131.1	100.0	20.8
Av.	.....	62.2	307.6	52.6	12.2	161.8	101.8	44.1

[a] Day before used as base value for calculations.

The chlorobutanol was placed directly in the rumen contents in crystalline form and was injected into the cecum and colon, dissolved in 10 c.c. of 95 per cent ethyl alcohol. As shown in table 8, the ascorbic acid content of blood and urine was followed daily throughout the trial. On the third and fourth days, blood samples were taken at 0, 1, 2, 4, and 24 hours to determine whether an immediate increase in blood ascorbic acid followed the administration of chlorobutanol. These results are shown in figure 3 and will be discussed later in this section.

As shown in table 8, blood ascorbic acid was not consistently affected during chlorobutanol treatment, but both whole blood and blood plasma ascorbic acid decreased significantly during the 3-day period following chlorobutanol administration. Urinary ascorbic acid was tripled by the fourth day of chlorobutanol treatment.

On the tenth and twelfth days (table 8) 16 g. of ascorbic acid were placed in the rumen and 4 g. in the colon and cecum; 2 g. of ascorbic acid were administered to each steer intravenously on the fourteenth day and 2 g. subcutaneously on the sixteenth day. These administrations were made

approximately 30 minutes after the daily dose of chlorobutanol. As shown in figure 3, the increases in blood ascorbic acid following the administration of ascorbic acid were not markedly affected by simultaneous chlorobutanol treatment. The maximum blood levels of ascorbic acid were observed 30 minutes after ascorbic acid injection rather than at 15 minutes when chloro-

TABLE 8

Administering chlorobutanol and ascorbic acid directly into various levels of the digestive tract

Day	Treatment[a]	Av. ascorbic acid of four steers[b]						
		Whole blood		Blood plasma		Urine		Recovery[d]
			Diff.[c]		Diff.[c]		Diff.[c]	
		(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)	(%)
1	None	0.490	.....	0.348	.....	89.9	.....	.....
2	None	0.438	.....	0.410	.....	72.0	.....	.....
3	Ch.[e]	0.435	−0.029	0.265	−0.114[f]	98.2	17.2	.....
4	Ch.	0.475	0.011	0.425	0.046	104.2	23.2	.....
5	Ch.	0.580	0.116[g]	0.438	0.059	153.8	72.8	.....
6	Ch.	0.482	0.018	0.322	−0.057	217.2	136.2	.....
7	Ch.	0.490	0.026	0.430	0.051	243.8	162.8[f]	.....
8	Ch.	0.558	0.094	0.390	−0.011	180.6	99.6	.....
9	Ch.	0.445	−0.019	0.322	−0.057	198.4	117.4	.....
10	Ch.[h]	0.488	0.024	0.355	−0.024	284.1	203.1[g]	0.9
11	Ch.	0.503	0.039	0.372	−0.007	180.0	99.0	.....
12	Ch.[h]	0.510	0.046	0.400	0.021	454.1	373.1[g]	2.6
13	Ch.	0.508	0.044	0.508	0.129[f]	142.6	61.6	.....
14	Ch.[i]	0.504	0.040	0.438	0.059	706.4	625.4[g]	25.8
15	Ch.	0.505	0.041	0.442	0.063	255.8	174.8[f]	.....
16	Ch.[j]	0.415	−0.049	0.295	−0.084	521.2	440.2[g]	15.3
17	Ch.	0.418	−0.046	0.270	−0.109[f]	192.5	111.5	.....
18	None	0.288	−0.176[g]	0.192	−0.187[g]	140.0	59.0	.....
19	None	0.368	−0.096	0.220	−0.159[g]	104.8	23.8	.....
20	None	0.565	0.101[f]	0.315	−0.064	206.1	125.1	.....
21	None	0.440	−0.024	0.382	−0.003	174.2	93.2	.....
22	None	0.588	0.124[f]	0.402	0.023	172.6	91.6	.....
23	None	0.525	0.061	0.410	0.031	107.7	26.7	.....

[a] Determinations made 24 hr. after each designated treatment.
[b] Represents two rumen, one colon, and one cecum fistula.
[c] Mean of days 1 and 2 used as base level.
[d] Mean of days 8 and 9 used as base level.
[e] Chlorobutanol.
[f] Significant mean difference—base levels compared to other means.
[g] Highly significant mean difference—base levels compared to other means.
[h] 4 g. ascorbic acid administered into cecum and colon, 16 g. into rumen.
[i] 2 g. ascorbic acid given intravenously.
[j] 2 g. ascorbic acid given subcutaneously.

butanol was not given; there was a tendency for the increased blood levels to be maintained longer.

The details of urinary elimination are shown in tables 8 and 9. Whereas in the previous trials digestive tract administration of ascorbic acid did not definitely increase urinary elimination, in these trials elimination, on the average, was 100 mg. higher for the first trial and 225 mg. higher for the

second trial as compared with the day prior to treatment. The percentage of administered ascorbic acid recovered on the day of treatment was calculated on the basis that urine excretion on the eighth and ninth days (table 8) was an acceptable base for ascorbic acid elimination in urine from steers receiving chlorobutanol. The amount recovered (stated in per cent of

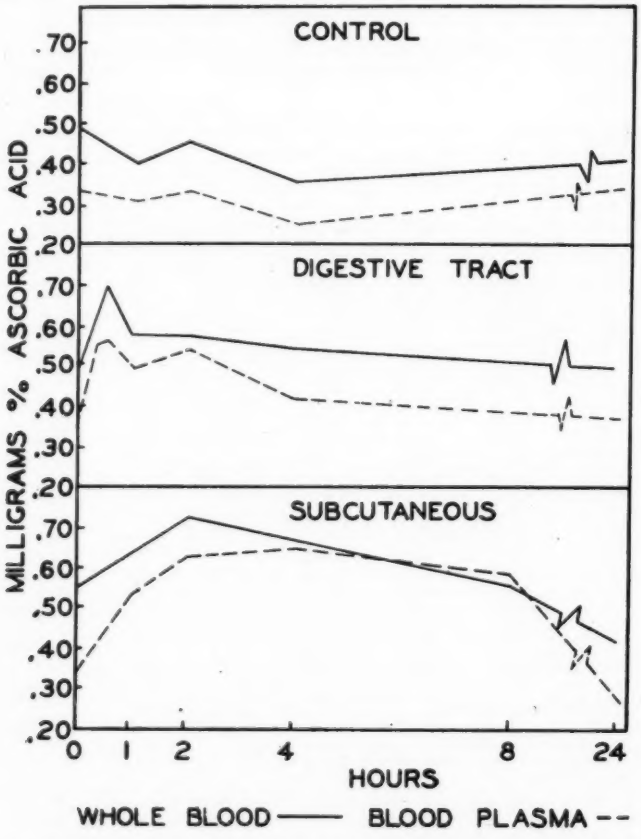

FIG. 3. The effect of chlorobutanol on blood ascorbic acid following ascorbic acid administration.

total administered) is shown by steers and by trials in table 9. The recovery from digestive tract administration still was quite low, since rather large quantities of ascorbic acid were given. The recovery following intravenous administration was 25.8 per cent as compared to 12.2 per cent when these same steers were not receiving chlorobutanol; recovery following subcu-

TABLE 9

Percentage of administered ascorbic acid recovered in the urine of steers receiving chlorobutanol

Steer	Percentage of ascorbic acid recovered after administration by way of:			
	Digestive tract[a]		Intravenous[b]	Subcutaneous[b]
	Trial 1	Trial 2		
	(%)	(%)	(%)	(%)
Colon	3.6	17.6	42.2	19.2
Cecum	0.4	2.6	20.6	4.9
Rumen—1	0.6	0.7	17.3	8.2
Rumen—2	0.8	0.9	23.2	29.0
Summary	0.9	2.6	25.8	15.3

[a] 4 g. of ascorbic acid placed in colon and cecum, 16 g. in rumens.
[b] 2 g. of ascorbic acid administered.

taneous administration also was 6 per cent higher than previously observed.

The data from this series of trials indicate that chlorobutanol increased urinary elimination of ascorbic acid approximately the same in all steers, even though administration was made in three different parts of the digestive tract, namely, rumen, colon, and cecum.

Subcutaneous administration of chlorobutanol. Base levels were established on the four steers used in the above studies. Three grams of chlorobutanol in 5 c.c. of 95 per cent ethyl alcohol then were injected subcutaneously into each steer daily for 5 days. Blood samples were taken daily and 24-hour urine specimens were collected. The results are shown in table 10. Whole blood and blood plasma ascorbic acid decreased considerably during the first 3 days of treatment. However, whole blood values

TABLE 10

Effect of administering chlorobutanol subcutaneously

Day	Treatment	Av. ascorbic acid of four steers					
		Whole blood		Blood plasma		Urine	
			Diff.[a]		Diff.[a]		Diff.[a]
		(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)	(mg.%)
1	None	0.380	.....	0.268	.....	71.2	.....
2	None	0.445	.....	0.330	.....	94.4	.....
3	Ch.[b]	0.368	−0.044	0.250	−0.049	84.1	1.3
4	Ch.	0.332	−0.080[c]	0.160	−0.130[d]	87.2	4.4
5	Ch.	0.285	−0.127[d]	0.225	−0.074	99.5	16.7
6	Ch.	0.432	0.020	0.262	−0.037	182.8	100.0[d]
7	Ch.	0.500	0.088[c]	0.265	−0.034	165.0	82.2[d]

[a] Mean of days 1 and 2 used as base level.
[b] Determination 24 hr. after treatment with chlorobutanol (Ch.).
[c] Significant mean difference—base levels compared to other means.
[d] Highly significant mean difference—base levels compared to other means.

increased for the next 2 days, while blood plasma remained slightly lower than for the first 2 days, which were used as base levels for purposes of comparison. Urinary excretion was nearly doubled on the fourth day after treatment began and remained at a high level on the last day of the trial. Each steer responded equally well each of the 2 days. This trial was discontinued as soon as a definite increase in urinary excretion was noted, since it was not thought desirable to give chlorobutanol in 95 per cent ethyl alcohol solution subcutaneously for a prolonged period. No indications of sloughing in the regions of administration were apparent several weeks afterwards.

Blood ascorbic acid relationships. Blood cell ascorbic acid was calculated for all blood samples using a blood cell volume-hemoglobin ratio determined by the random selection of two blood samples from each of four steers. This ratio on 48 such samples averaged 2.145 ± 0.035. Hemoglobin determinations were made on all samples. Correlation and regression coefficients were determined between whole blood and blood plasma ascorbic acid values for the various bleeding intervals and treatments reported in this study. The original data for these studies are reported elsewhere (4). Results indicate that cellular elements of the blood are permeable to ascorbic acid. This was particularly apparent following intravenous administration. However, in all these trials (tables 1 and 2, figs. 1 and 2), whole blood ascorbic acid tended to increase more slowly and decrease less rapidly than blood plasma ascorbic acid. Calculated blood cell ascorbic acid varied considerably and seemed to have a cyclic rate of change. For example, in the case of intravenous administration, calculated blood cell ascorbic acid was quite high at 1, 2, and 4 minutes after treatment and then decreased in the next 12 minutes to considerably below base levels, slowly recovering thereafter. The magnitude of this phenomenon was not so pronounced in the other types of administration. The blood cell ascorbic acid content for these steers usually varied from approximately 0.7 to 1.0 mg. per cent during non-treatment periods but consistently showed values above and below during experimental periods. This indicates that the ascorbic acid balance between plasma and cells was upset and that some compensatory action was taking place.

Interval of bleeding on the hemoglobin content of blood. Hemoglobin values were observed to decrease rather uniformly during periods when blood samples were drawn frequently. Some of these data were summarized for the various treaments and various bleeding intervals. The results, as shown in table 11, reveal that hemoglobin was reduced significantly during the periods of rapid bleeding used in the control, digestive tract (rumen, colon, and cecum summary), small intestine, and intravenous studies. This drop in hemoglobin also has been observed recently in sheep by Thomson *et al.* (29). The *F*-ratio between bleeding intervals was highly

significant. The bleeding intervals used during the subcutaneous studies did not show any apparent influence on the hemoglobin content of blood,

TABLE 11

Effect of bleeding interval on average hemoglobin in the blood of steers

Bleeding interval	No. of sample series	Av. Hb. (*gm.%*)	Diff.[a] (*gm.%*)
Control studies			
−24 hr.	8	13.94	.....
0		13.18	.....
15 min.		12.96	−0.75
30 min.		12.33	−1.38[b]
60 min.		12.78	−0.93[c]
90 min.		12.15	−1.56[b]
2 hr.		11.88	−1.83[b]
3 hr.		13.19	−0.52
4 hr.		14.42	0.71
6 hr.		13.42	−0.29
8 hr.		13.60	−0.11
24 hr.		14.01	.....
Digestive tract studies[d]			
−24 hr.	32	13.17	.....
0		13.60	.....
15 min.		13.58	0.41
30 min.		13.00	−0.17
60 min.		12.75	−0.42
90 min.		12.74	−0.43
2 hr.		12.30	−0.87[b]
3 hr.		12.76	−0.41
4 hr.		13.25	−0.08
6 hr.		12.61	−0.56[c]
24 hr.		12.74	.....
Small intestine studies			
−24 hr.	8	10.84	.....
0		11.03	.....
15 min.		9.67	−1.36[b]
30 min.		10.43	−0.60
60 min.		9.80	−1.23[b]
90 min.		8.99	−2.04[b]
2 hr.		9.28	−1.75[b]
3 hr.		9.86	−1.17[b]
4 hr.		9.70	−1.33[b]
6 hr.		9.93	−1.10[b]
24 hr.		11.20	.....
Subcutaneous studies			
−24 hr.	8	12.62	.....
0		13.51	.....
1 hr		13.02	−0.27
2 hr.		13.53	0.24
3 hr.		13.42	0.13
4 hr.		13.22	−0.07
6 hr.		13.46	0.17
8 hr.		12.91	−0.38
24 hr.		13.46	.....
Intravenous studies			
−24 hr.	8	13.34	.....
0		14.46	.....
1 min.		13.70	0.27
2 min.		13.94	0.51
4 min.		14.04	0.61
8 min.		13.76	0.33
16 min.		12.57	−0.86
32 min.		13.48	0.05
60 min.		13.15	−0.28
90 min.		13.03	−0.40
2 hr.		12.32	−1.11[c]
3 hr.		12.29	−1.15[c]
4 hr.		11.16	−2.27[b]
24 hr.		12.46	.....

[a] Mean of −24, 0, and 24 hr. used as base level.
[b] Highly significant mean difference.
[c] Significant mean difference.
[d] Four steer summary includes rumen 1 and 2, colon, and cecum.

the *F*-ratio being only 0.34. Since feeding and watering were done at the same relative time in all the studies, variation due to these factors should have been apparent in the subcutaneous trials. Furthermore, the steers drank

very little water after the morning feeding; maximum water intake invariably took place after the evening feeding.

DISCUSSION

Absorption of ascorbic acid from the digestive tract. Riddell *et al.* (22) changed three dairy cows abruptly from a winter ration to one consisting of 65 kg. of green rye daily. They observed that ascorbic acid in blood plasma was more than doubled during the first 12 hours and that urine output increased five times within 60 hours. Later, Knight *et al.* (10) and Vavich *et al.* (32) fed or administered up to 150 g. of ascorbic acid to Holstein cows without observing any increases in blood plasma ascorbic acid in samples taken 1.5 hours after administering the vitamin. Vavich *et al.* (32) also fed dehydrated cereal grasses to one cow without influencing blood plasma ascorbic acid. Lundquist and Phillips (13) observed that ascorbic acid administered *per os* to calves would raise blood plasma levels for the first 10–12 days of life but was ineffective thereafter.

In this study a total of 76 trials were conducted on five steers. The administration of 16 g. of ascorbic acid into the ventral rumen sac significantly increased whole blood and blood plasma ascorbic acid within 15 minutes. Slight increases were noted for the first 4 hours, but these mean differences were not statistically significant. The administration of 4 g. of ascorbic acid into the cecum and colon also produced significant increases in blood ascorbic acid during the first 2 hours following treatment. The maximum values, however, generally were observed within the first 15 minutes. When the 32 trials (representing 12 rumen, 10 cecum, and 10 colon) were summarized, significant mean differences in whole blood and blood plasma ascorbic acid were observed at 15, 30, 60, and 90 minutes, and at 2 and 3 hours after administration of ascorbic acid. When 50 c.c. of distilled water were administered to these steers (8 trials) and the same bleeding routine followed, whole blood ascorbic acid was significantly decreased after 1, 1.5, and 4 hours, while blood plasma ascorbic acid was significantly lowered at 4 hours. Average urine elimination of ascorbic acid was significantly higher during the 24-hour period following treatment when 16 g. of ascorbic acid were placed in the rumen, but little or no increase was observed from placing 4 g. of the vitamin in the colon or cecum.

The administration of 4 g. of ascorbic acid into the small intestine also significantly increased ascorbic acid in whole blood and blood plasma for the first hour after treatment, with the maximum increase coming at 30 minutes.

The deposition of 16 g. of ascorbic acid in the reticulo-omasal-abomasal orifice also significantly increased ascorbic acid in whole blood and blood plasma and more than tripled urine elimination of ascorbic acid. Holding ascorbic acid against the rumen wall significantly increased whole blood and

blood plasma ascorbic acid. Administration of 16 g. of ascorbic acid by stomach tube increased blood ascorbic acid in three out of six trials, but these increases were not significant. Likewise, urinary elimination was not noticeably increased by this treatment.

Though results in these studies are not in agreement with Knight *et al.* (10) and Vavich *et al.* (32), a direct comparison cannot be made. Maximum absorption of ascorbic acid in this study was observed within 15 minutes after treatment, whereas, Knight *et al.* (10) and Vavich *et al.* (32) apparently did not take blood samples prior to 1.5 hours after feeding or placing ascorbic acid in the rumen. The time lag between administration and bleeding could explain the failure to observe increased blood plasma ascorbic acid. However, these authors did observe slight increases in urine elimination of ascorbic acid following treatment.

Daily administration of 5 g. of chlorobutanol for 15 days into the rumen, colon, and cecum tripled the urinary output of ascorbic acid by the fifth day. This increase essentially was the same as that observed in dairy cattle by Moore and Cotter (17) and Moore (16), and in rats by Longenecker *et al.* (12) and Sutton *et al.* (28). The ascorbic acid content of whole blood and blood plasma was not increased. This may have been due to the short administration period, since Scheidenhelm *et al.* (26), Bortree *et al.* (2, 3), Lundquist and Phillips (14, 15), Moore and Cotter (17), and Moore (16) frequently observed animals that required chlorobutanol administration for more than 15 days before blood plasma ascorbic acid was raised. A pronounced drop in blood ascorbic acid occurred for several days immediately following the cessation of chlorobutanol treatment. This also was observed by the previous workers, but their observed decrease was not so rapid and recovery was much slower.

Ascorbic acid, when administered subcutaneously, intravenously, or into the respective fistulae of these chlorobutanol-conditioned steers, resulted in a marked increase in the per cent of administered ascorbic acid recovered as compared to recovery from the same steers when not receiving chlorobutanol. Ascorbic acid in the urine increased, on the average, 100 to 200 mg. when ascorbic acid was placed in the digestive tract following chlorobutanol treatment as compared to much smaller increases when the steers were not receiving chlorobutanol. Intravenous recovery of ascorbic acid was increased from 12.2 to 25.8 per cent and subcutaneous recovery from 9.3 to 15.3 per cent by chlorobutanol treatment. The quantities of ascorbic acid found in venous blood did not seem to be appreciably greater. However, peak values generally were observed at 30 instead of 15 minutes. Knight *et al.* (10) gave 24 g. of ascorbic acid to cows subcutaneously and intravenously for 3 successive days. They recovered about 25 per cent of the ascorbic acid in the case of intravenous administration and slightly less in subcutaneous administration, according to our calculations from their

original data. In the present study only 2 g. of ascorbic acid were used and for only 1 day. The magnitude of recovery was less in this case except when administration was made to chlorobutanol-conditioned animals. The low rate of recovery of ascorbic acid in urine, even when the vitamin was placed directly in the blood stream at the rate of 24 g. per day for 3 successive days (10) suggests that the bovine does not readily eliminate ascorbic acid in the urine. Several possibilities exist: the vitamin could be eliminated in feces, it could be absorbed by other body tissues, or it could be used as a metabolite. Feces studies (4) do not suggest that any appreciable fecal elimination takes place. Ascorbic acid also could be oxidized to the diketogulonic acid or other non-reversible oxidative forms and thus not be detected by the methods of analysis used. The extensive studies of Borsook *et al.* (1) show quite clearly that ascorbic acid is quite stable in blood and tissues and that dehydroascorbic acid is reduced rapidly in minced tissues. This reducing substance further was shown to be glutathione. This does not rule out the possibility that, when excessive amounts of ascorbic acid suddenly are placed in the blood stream, the non-reversible oxidative forms would not be produced. It seems more than probable, however, that the animal body can use excess quantities of ascorbic acid as a metabolite. It would be difficult to determine how much ascorbic acid was absorbed from digestive tract administration. The mechanism by which chlorobutanol causes increased ascorbic acid elimination in urine and raises blood levels is unknown. Whether the fact that recovery of administered ascorbic acid is markedly increased in steers receiving chlorobutanol adds anything to the problem is difficult to say. If the theory is accepted that body synthesis is increased, then increased urinary elimination of administered ascorbic acid could be explained by the fact that the body has less use for the extra ascorbic acid, which then would be eliminated. On the other hand, chlorobutanol may merely decrease the ability of the organism to utilize the vitamin and in this manner cause increased ascorbic acid elimination. Feng (6) reported that feeding chlorobutanol to rats increased the ascorbic acid content of adrenals, kidneys, intestine and liver over the controls. This would suggest that ascorbic acid is not driven out of the tissues. The observations by Moore and Cotter (17) that pregnant heifers on a low carotene ration excreted more ascorbic acid in the urine than pregnant heifers fed normally and that such heifers did not show any further increase when fed chlorobutanol also are of speculative interest.

When the digestive tract is by-passed by administering chlorobutanol subcutaneously, urinary elimination of ascorbic acid is increased as rapidly as when the drug was placed in the digestive tract. No apparent changes occurred in blood ascorbic acid during the 5-day treatment period.

The rate of removal of ascorbic acid from the blood stream. Bleeding steers 10 times in a single 8-hour period significantly reduced the ascorbic acid content of whole blood and blood plasma ascorbic acid. Increases in

blood ascorbic acid resulting from administering the vitamin directly into the digestive tract were frequently noted at 15 and 30 minutes after treatment but not at 60 minutes or at any time thereafter. Subcuaneous administration of 2 g. of ascorbic acid was followed by maximum blood levels in 3 to 6 hours, with return to base levels at 24 hours. When 2 g. of ascorbic acid were given intravenously, approximately one-half was removed from venous blood in 4 minutes and half of the remainder in the next 12 minutes, and this again was reduced by half at the end of 32 minutes. While somewhat higher levels of blood ascorbic acid were observed 4 hours after administration as compared to the base period, these values had returned to base levels after 24 hours.

Blood cell and blood plasma ascorbic acid relationships. Borsook *et al.* (1) studied permeability of red blood cells by adding ascorbic acid to oxalated whole blood and incubating for 1 hour at 37.5° C. They concluded that the red corpuscles of beef cattle, cats, dogs, humans, pigs, rats and sheep are nearly, if not absolutely, impermeable to added ascorbic acid. There were some slight indications, however, that the red corpuscles of pigs and sheep had some permeability.

Results in this study indicate that the cellular elements of the blood do absorb some ascorbic acid. This was particularly apparent during the first 4 minutes following intravenous administration. It also was noted during subcutaneous and digestive tract administration. However, in all these trials, whole blood ascorbic acid tended to increase more slowly and decrease less rapidly than blood plasma ascorbic acid. Calculated blood cell ascorbic acid varied considerably and seemed to have a cyclic rate of change. For example, in the case of intravenous administration, calculated blood cell ascorbic acid was quite high at 1, 2, and 4 minutes after treatment and then decreased in the next 12 minutes to considerably below base levels, and then recovered slowly thereafter.

SUMMARY

1. The ascorbic acid content of whole blood and blood plasma was significantly increased within 15 minutes after placing ascorbic acid into the rumen, reticulo-omasal-abomasal orifice, small intestine, cecum, and colon. These increases persisted for only 2–3 hours.

2. Holding ascorbic acid solution against the rumen wall significantly increased the ascorbic acid content of whole blood and blood plasma.

3. Very little ascorbic acid was recovered in the urine following digestive tract administration, and only 9.2 and 12.3 per cent, respectively, were recovered following subcutaneous and intravenous administration. The recovery of ascorbic acid was greatly increased when these types of administration were made to steers receiving chlorobutanol.

4. The administration of chlorobutanol into the rumen, colon, and cecum at the rate of 5 g. daily for 15 days or subcutaneous administration at the rate of 3 g. daily for 5 days significantly increased urinary elimination

of ascorbic acid the fourth day after the start of treatment. Blood ascorbic acid was not increased by either method of chlorobutanol administration.

5. Calculations made to determine the ascorbic acid content of blood cells indicated that the vitamin passed freely between blood cells and blood plasma, although in the case of subcutaneous or intravenous administration blood plasma values were at times considerably higher than for whole blood.

6. The hemoglobin content of blood was significantly decreased by bleeding cattle at frequent intervals.

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THE PHARMACODYNAMICS OF ASCORBIC ACID IN CATTLE. II. *IN VIVO* AND *IN VITRO* LOSS[1, 2]

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Investigations by Whitnah and Riddell (13), Knight *et al.* (6), and Vavich *et al.* (11) have shown that ascorbic acid rapidly is lost in the rumen contents of cattle both *in vivo* and *in vitro*. Kendall and Chinn (4) were unable to show *in vitro* decomposition of ascorbic acid in filtered rumen juice obtained from freshly slaughtered cows. Knight *et al.* (6) found that ascorbic acid rapidly disappeared from the rumen contents of a cow (*in vivo*) after she was fed as much as 150 g. of crystalline ascorbic acid. Studies of rumen juice incubated at 39–42° C. revealed that loss of added ascorbic acid was rapid but not as rapid as *in vivo*. This was attributed to lack of continuous mixing *in vitro* compared to continuous mixing *in vivo*.

Vavich *et al.* (11) found that ascorbic acid was destroyed quite slowly in sterilized rumen contents. When dehydrated cereal grasses were fed as a source of ascorbic acid, the vitamin could be demonstrated in the rumen more than 8 hours after feeding, but the highest concentration occurred 2 to 4 hours after feeding. Orally administered crystalline ascorbic acid could be recovered for approximately 2 hours. These authors also removed all rumen contents, flushed the rumen with warm water, and again emptied it. The cow then was fed a low ascorbic acid ration. *In vivo* and *in vitro* studies of this material showed little loss of ascorbic acid, but when 2 gallons of the fermenting rumen contents were returned to the rumen, definite loss of added ascorbic acid occurred. Young and James (14) and Young and Rettger (15) have reported that human fecal cultures and isolated cultures of *Escherichia coli* and *Aerobacter aerogenes* decomposed ascorbic acid under both aerobic and anaerobic conditions. Organisms of the *Alcaligenes* and *Proteus* groups did not attack ascorbic acid and protected it from atmospheric oxidations but not from bacteria decomposing ascorbic acid. Glucose and lactose "spared" ascorbic acid from oxidation by fecal bacteria. Ascorbic acid-decomposing bacteria which produced gas from ordinary carbohydrates also produced gas when attacking ascorbic acid. Stepp and Schroder (10) also have reported on a strain of *Escherichia coli* that decomposed ascorbic acid.

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The pH in various parts of the digestive tract is quite variable. Dukes (1) reports that most rumen studies of cattle show a range of pH 6.0 to 7.8. Kick *et al.* (5), working with steers, reported that their determinations varied with rations fed, ranging from pH 5.5 to 7.7, with the most alkaline reaction being on material from animals fed alfalfa alone. Monroe and Perkins (7) reported an average pH value between 6.83 and 7.01 for animals fed such roughages as corn or A.I.V. silage and alfalfa hay. They found that when samples were taken at 2-hour intervals throughout the day under dry lot conditions, the pH was most alkaline just before the morning feeding, with the largest variations occurring just after feeding. Smith (9) made *in vivo* studies on two cows and found that rumen pH was 6.30 on an alfalfa ration and 6.07 on alfalfa and beet pulp. The pH generally was more alkaline shortly before and after feeding and varied from 6.27 in the front to 6.00 in the rear of the rumen.

The available literature on rumen flora and fauna has been excellently reviewed by Hastings (3). In general, numbers of bacteria have been difficult to estimate, since many do not grow on the usual laboratory media. Protozoa concentrations have been reported variously from 500 million to 1 billion per ml. These organisms have been estimated to comprise as much as 10 per cent of the total dry matter in the rumen.

EXPERIMENTAL

Data on the rate of removal or loss of ascorbic acid from the contents of various portions of the digestive tract were gathered simultaneously with the data on absorption and elimination of ascorbic acid, which were reported in the previous paper (2). Rumen juice, small intestinal juice, cecal and colon contents, and the feces were studied. Ascorbic acid was determined by the method of Roe and Keuther (8) with such modifications as were necessary to give satisfactory dilutions. All colorimetric determinations were made with the Evelyn photoelectric colorimeter. All pH determinations were made with a glass electrode pH meter.

Samples of rumen juice were taken from the ventral rumen sac. Cecal and colon contents were allowed to ooze from the fistulae into 50 c.c. flasks. These samples were taken immediately to the laboratory for pH and ascorbic acid determinations.

The ascorbic acid content and pH of the digestive tract contents before and following the administration of ascorbic acid are shown in table 1. The pH range of 12 determinations on the rumen contents was 5.72 to 6.56, and 6.92 to 8.12 in the colon in untreated animals. The administration of 16 g. of ascorbic acid in the ventral rumen sac did not lower the pH of the rumen juice of the two steers. When the ascorbic acid level of the colon contents was above 300 mg. per cent, pH appeared to be lowered in several instances and ranged between 5.88 and 7.76. The contents from the cecal fistula did

TABLE 1

Ascorbic acid recovery in vivo

Administration		Av. and no. of cases	Hr. from treatment											
			−24	0	0.25	0.5	1	1.5	2	3	4	6	8	24
(*site*)	(*g.*)													
Rumen—2	0	No.		2	2	2	2	2	2					2
		A.A.[a] mg.%		0.12	0.10	0.03	0.06	0.04	0.06					0.22
		pH		6.10	6.23	6.10	6.44	6.49	6.32					6.10
Colon	0	No.		2	2	2	2	2	2					2
		A.A.mg.%		0.28	0.78	0.25	0.55	0.40	0.13					0.40
		pH		7.42	7.66	7.28	7.41	7.31	7.52					7.29
Rumen—1	16	No.	2	4	3	4	5	3	4	4	4	2		3
		A.A.mg.%	1.28	1.21	95.0	30.0	19.0	1.62	1.09	1.26	1.12	0.14		0.91
		pH	6.45	6.80	6.72	6.88	6.96	6.51	6.87	6.68	6.55	7.18		6.34
Rumen—2	16	No.	2	4	3	4	5	3	4	4	4	2		3
		A.A.mg.%	1.20	1.02	194.9	135.0	42.2	55.0	15.2	1.24	0.90	0.16		0.82
		pH	6.46	6.72	6.56	6.50	6.75	6.43	6.82	6.51	6.57	6.35		6.44
Rumens—1–2		A.A.—range	0.49–1.90	0.27–1.80	63.7–407.0	21.0–317.0	0.39–133.0	0.77–95.0	0.24–59.0	0.23–2.50	0.20–1.90	0.11–0.21		0.06–1.90
		pH—range	6.28–6.64	6.34–7.38	6.34–6.84	6.24–7.38	6.24–7.46	5.74–6.94	6.24–7.20	6.08–7.18	6.08–7.16	6.18–7.24		6.12–6.64
Colon	4	No.	4	5	5	5	7	5	5	5	6	4	4	5
		A.A.mg.%	2.10	2.39	268.7	37.1	38.3	21.7	59.1	3.80	3.88	1.78	0.62	1.70
		Range	0.10–5.9	0.28–7.2	32.4–693.0	4.6–283.0	3.7–96.4	2.4–54.9	2.5–166.2	1.2–6.1	0.81–6.0	0.99–3.2	0.38–0.93	0.12–5.90
		No.	2	4	4	3	5	5	3	2	5	1	1	3
		pH	7.20	7.31	6.82	7.35	7.15	7.12	7.33	6.89	6.91	7.02	7.42	7.0
		Range	7.64–6.76	6.98–7.52	5.88–7.54	7.04–7.64	6.82–7.48	6.64–7.60	7.08–7.46	6.84–6.94	6.72–7.32			6.64–7.76
Cecum	4	No.	1	3	4	2	3	2	3	2	4	1	3	3
		A.A.mg.%	0.21	1.15	327.7	264.8	301.5	38.8	29.4	158.5	71.3	8.4	3.8	9.3
		Range		0.60–2.11	29.4–528.0	28.4–501.2	111.0–573.8	18.1–59.5	3.4–62.5	120.3–196.8	9.4–112.8		0.80–1.90	0.21–27.4
		No.		1	2		2		2	1	2			1
		pH		6.70	6.72		6.83		7.42	7.12	7.73			7.26
		Range			6.46–6.97		6.74–6.92		7.02–7.82		7.02–8.45			

[a] A.A. = Ascorbic acid.

not ooze from the opening at a constant rate, and fewer determinations were made from this area. The pH range following ascorbic acid treatment was 6.46 to 8.45. Considerable quantities of ascorbic acid were recovered in the rumen juice during the first 2 hours following administration of 16 g. and in the colon contents during the first 4 hours after injecting 4 g. of ascorbic acid. In the cecum appreciable amounts of ascorbic acid were present 24 hours after administration.

The effects of subcutaneous and intravenous administration of ascorbic acid upon the amount of ascorbic acid in the digestive tract contents are shown in table 2. Ascorbic acid treatment by these routes had no apparent effect on either ascorbic acid content or pH. The administration of chlorobutanol into the rumen, colon, and cecum at the rate of 5 g. daily for 9 days had no effect on pH or ascorbic acid content of the ingesta.

The ascorbic acid content of freshly voided feces was determined prior to treatment and following administration of ascorbic acid and chlorobutanol. These findings are summarized in table 3. The administration of ascorbic acid into the rumen, cecum, and colon appeared to increase fecal elimination of ascorbic acid. Large quantities of ascorbic acid appeared in the feces 8 hours after administration into the cecum. This was not so apparent in the case of the rumen- and colon-fistulated steers, although fecal ascorbic acid appeared to be higher up to 24 hours. Possibly the sampling time of 8 hours after treatment was too long in the case of the colon and too short an interval after rumen administration. However, oxidation and dilution of ascorbic acid placed in the rumen would be expected to decrease greatly the possibility of such treatment increasing fecal ascorbic acid. The subcutaneous and intravenous administration of ascorbic acid had little if any effect on the feces. Chlorobutanol, when placed in the various fistulae, had no apparent effect on the ascorbic acid content of the feces.

A few determinations were made on small intestinal contents. These results are shown in table 4. Results of four determinations made before treatment with 4 g. of ascorbic acid showed ascorbic acid to be no higher than was found in rumen juice. The pH, however, was quite alkaline, ranging from 7.22 to 8.16 on 12 samples and averaging 7.63. Considerable ascorbic acid was found in the contents 1.5 hours after administration of the vitamin. Samples were not taken sooner because of the necessity of keeping the fistula-lumen plugged for this period of time after administration to prevent loss of the vitamin. Administration also was made posteriorly into the gut, so that most of the ascorbic acid probably passed too far back to leak out of the fistula when ingesta samples were taken.

Rate of loss of ascorbic acid added in vivo and incubated in vitro at 37° C. In the previous section it was shown that samples of digestive tract contents removed at regular intervals after administration of ascorbic acid contained appreciable quantities of the vitamin for at least 2 hours. To determine the

TABLE 2

The effect of administering ascorbic acid, subcutaneously and intravenously, and chlorobutanol into the digestive tract

Treatment		Av. and range	Steer			
			Colon	Cecum	Rumen—1	Rumen—2
(*type*)	(*g.*)					
Ascorbic acid subcutaneously	2	A.A.[a]mg.%	0.69 (17)[b]			0.24 (16)
		Range	0–1.68			0.04–0.75
		pH	7.59 (14)			6.60 (16)
		Range	7.02–8.00			6.00–7.26
Ascorbic acid intravenously	2	A.A.mg.%	0.57 (8)			0.29 (8)
		Range	0.23–0.95			0.04–1.35
		pH	7.26 (5)			6.39 (8)
		Range	6.86–7.52			5.88–6.52
Chlorobutanol in digestive tract	5	A.A.mg.%	0.64 (10)	0.62 (4)	0.19 (11)	0.31 (11)
		Range	0.30–0.88	0.15–1.12	0–0.45	0–1.50
		pH	7.15 (8)	6.85 (2)	6.94 (9)	6.83 (9)
		Range	6.86–7.52	6.68–7.02	6.70–7.08	6.48–7.12

[a] Ascorbic acid.
[b] Figures in parentheses indicate number of determinations.

TABLE 3

The effect of administering ascorbic acid and chlorobutanol on the ascorbic acid content of the feces

Treatment		Time interval	Av. and range of ascorbic acid	Steer			Summary[a]
				Colon	Cecum	Rumen—1–2	
(*type*)	(*g.*)	(*hr.*)		(*mg.%*)	(*mg.%*)	(*mg.%*)	(*mg.%*)
Ascorbic acid in digestive tract	4 and 16[b]	0	Av.	1.12 (3)[c]	2.40 (3)	2.16 (6)	
			Range	0.18–2.00	0.59– 3.80	0.70– 3.90	
		8	Av.	2.17 (3)	43.7 (3)	4.55 (5)	
			Range	1.32–2.80	25.0 –70.8	2.10–10.60	
		24	Av.	2.38 (2)	2.04 (2)	1.58 (4)	
			Range	0.47–4.30	0.68– 3.40	0.43– 2.80	
Ascorbic acid subcutaneously	2	0	Av.				0.854 (8)
			Range				0.60–1.08
		8	Av.				0.652 (8)
			Range				0–1.60
		24	Av.				0.365 (4)
			Range				0.23–0.51
Ascorbic acid intravenously	2	0	Av.				1.20 (4)
			Range				0.64–1.92
		4	Av.				1.78 (4)
			Range				1.12–2.38
Chlorobutanol in digestive tract	5	Daily	Av.	0.80 (7)	0.69 (8)	0.68 (15)	0.712 (30)
			Range	0.47–1.30	0.24–0.98	0–1.80	0–1.80

[a] Same number of samples from each steer.
[b] 4 g. in colon and cecum; 16 g. in rumen.
[c] Figures in parentheses indicate number of determinations.

TABLE 4

In vivo recovery of ascorbic acid from a jejunal fistula and the pH value of the contents

Av. and range	Time interval—hr. after treatment				
	0	1.5	2	3	24
A.A.mg.%	0.28 (4)[a]	28.7 (4)	0.70 (3)	0.78 (1)	0.54 (3)
Range	0.19–0.34	9.0 –43.1	0.23–0.98		0.28–0.73
pH	7.65 (3)	7.65 (3)	7.62 (3)	7.16 (1)	7.84 (2)
Range	7.54–7.86	7.22–8.02	7.32–7.92		7.52–8.16

[a] Figures in parentheses indicate number of determinations.

rate of loss of ascorbic acid in ingesta outside the body, trials were conducted on contents from the small intestine, colon, and rumen following administration of ascorbic acid into these regions.

Samples of the rumen, small intestinal, and colon contents were collected in flasks, tightly stoppered except during sampling, and pH and ascorbic acid determinations were made immediately. The samples then were placed in a water bath at 37° C. and ascorbic acid determinations made after 2, 4, 6, 11, and 23 hours of incubation. The colon and rumen contents were obtained from the fistulae 15 minutes after the administration of 4 and 16 g. of ascorbic acid into the colon and rumen, respectively, and the small intestinal contents were secured 1.5 hours following the injection of 4 g. of the vitamin.

The results are shown in figures 1 and 2. Two widely different concentrations of ascorbic acid were secured for the incubation studies on small intestinal contents. One series of four trials showed an average of 42.6 mg. per cent ascorbic acid prior to incubation; after 6 hours of incubation approximately one-half of the ascorbic acid remained. Half of the remainder was lost in the next 5 hours. The other series of four trials had only 2.8 mg. per cent ascorbic acid prior to incubation and lost, on the average, approximately one-half of the ascorbic acid in 4 hours.

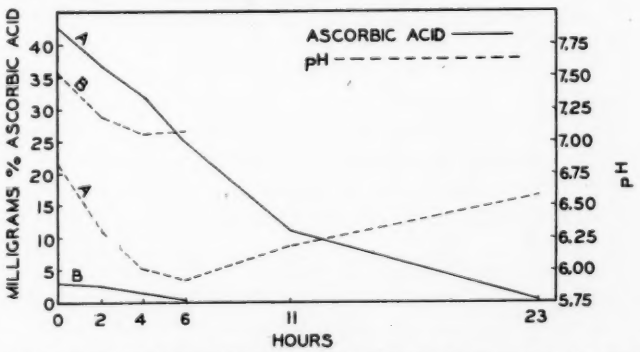

FIG. 1. *In vitro* loss of ascorbic acid and pH change in contents of the small intestine incubated at 37° C.—ascorbic acid added *in vivo*.

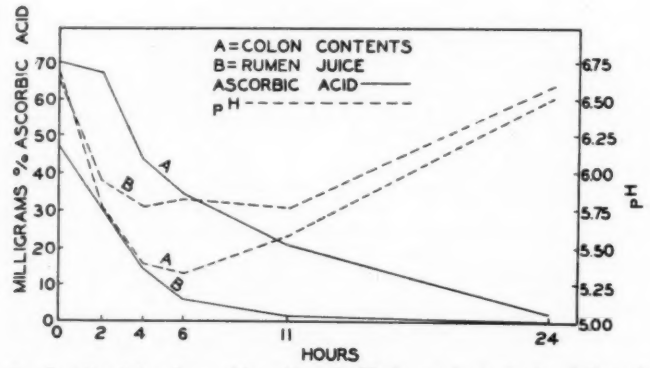

FIG. 2. *In vitro* loss of ascorbic acid and pH change in contents of the colon and rumen incubated at 37° C.—ascorbic acid added *in vivo*.

The average rate of loss of ascorbic acid from colon contents was approximately the same as for small intestinal contents. More ascorbic acid was left after 11 hours of incubation, but considerably more was present prior to incubation. The rate of loss of ascorbic acid from rumen juice, as shown in figure 2, was essentially twice as rapid as that in small intestinal and colon contents. The pH in all cases dropped for the first 4 to 6 hours and then gradually increased throughout the remainder of the incubation period.

Results from the previous section indicated that ascorbic acid gradually was lost from digestive tract contents over a period of 11 to 23 hours, the length of time depending on the amount originally in the incubated sample. Since it was not possible to get samples of uniform concentration from the *in vivo* method of mixing, ascorbic acid was added to rumen juice and colon contents in the laboratory. Samples of the rumen, small intestinal, and

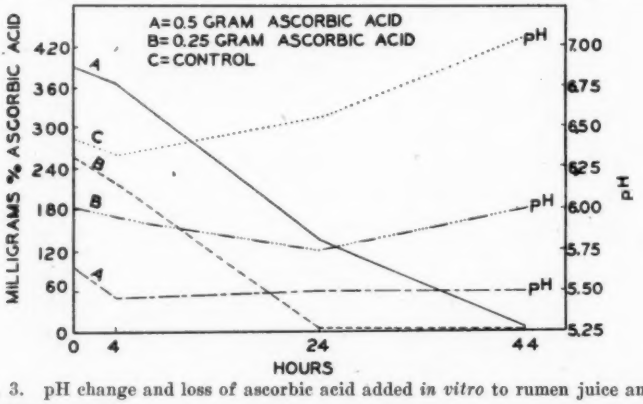

FIG. 3. pH change and loss of ascorbic acid added *in vitro* to rumen juice and incubated at 37° C.

colon contents were collected and divided into three portions of 100 c.c. each. One portion was used as a control, 0.25 g. of ascorbic acid was added to another, and 0.5 g. of ascorbic acid to the third. The ascorbic acid was mixed into the contents by shaking in tightly stoppered bottles, and a sample for pH and ascorbic acid determinations was removed and the remainder placed in a water bath at 37° C. The results of six trials on rumen juice and four trials on colon contents are shown in figures 3 and 4. The amount of ascorbic acid determined after addition of ascorbic acid and at the start of incubation was not any higher than quantities that occasionally were recovered from the digestive tract after vitamin administration. The average rate of loss was surprisingly constant for the first 24 hours in the rumen juice for

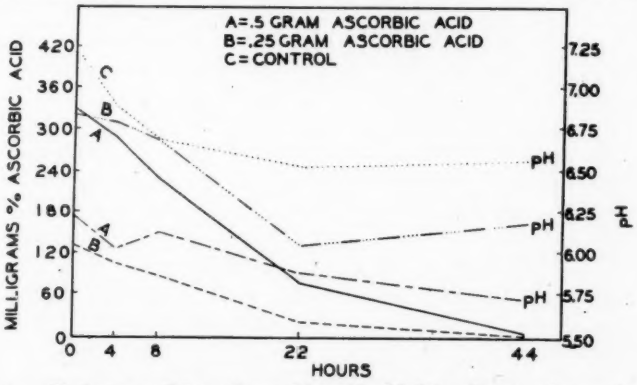

FIG. 4. pH change and loss of ascorbic acid added *in vitro* to colon contents and incubated at 37° C.

both the high and low ascorbic acid samples, approximately 250 mg. of ascorbic acid being lost in each case. The average rate of loss of ascorbic acid in colon contents was slightly higher than the loss in rumen juice during the first 22 hours in the sample containing 0.5 g. of ascorbic acid. However, the colon samples which contained 0.25 g. of ascorbic acid showed an average loss approximately one-third as great; after 44 hours of incubation the amount of ascorbic acid remaining was, on the average, nearly the same in the two samples. The pH was lowered considerably by the addition of ascorbic acid. This decrease was greater than in the case of *in vivo* samples taken after ascorbic acid administration, even though the amount of ascorbic acid in these *in vivo* samples was in some cases higher than the concentration in the *in vitro* samples.

The effect of adding starch, autoclaving, and filtering rumen juice on loss of added ascorbic acid. Rumen juice samples for this study were taken from a Jersey cow with a rumen fistula during the last week of May, 1946. This cow was maintained on bluegrass pasture and received no grain. Approxi-

mately 1 l. of rumen juice was removed for each trial. Samples were prepared for incubation in screw capped jars and ascorbic acid was added at the rate of 4 mg. per 100 c.c. of material, except in the case of the bacteria-free filtrate, in which ascorbic acid was added at the rate of 8 mg. per 100 c.c. of material.

Corn starch was added at the rate of 50 g. per l. of ingesta. Samples were autoclaved at 15 lb. pressure for 30 minutes. Approximately 50 c.c. of bac-

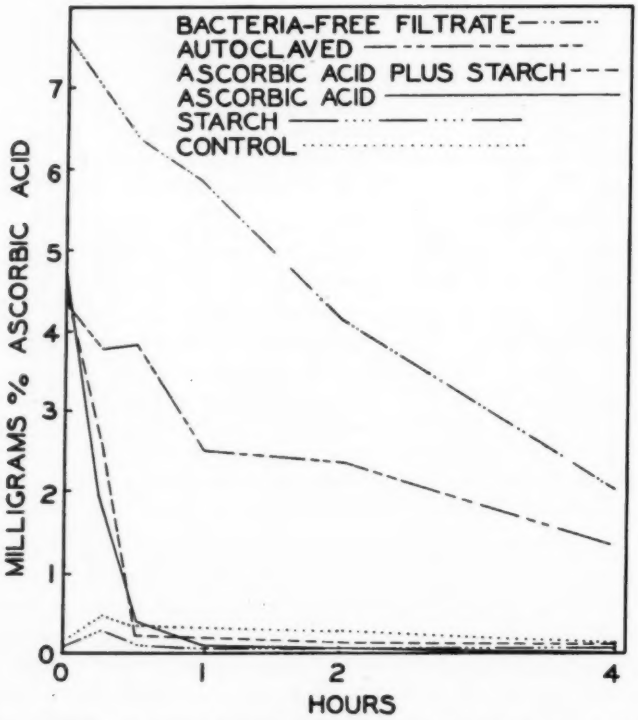

FIG. 5. The effects of starch, autoclaving, and removal of bacteria on rate of loss of ascorbic acid *in vitro* in rumen juice incubated at 37° C.

teria-free filtrate were prepared by filtering through cotton and then through a Seitz filter. Fifty milligrams of chlorobutanol and thiourea were added to some samples singly and together. These two materials had no effect on the rate of loss of added ascorbic acid. The factors studied, as shown in figure 5, were starch added *vs.* no starch, ascorbic acid alone *vs.* ascorbic acid and starch, autoclaving plus ascorbic acid, and bacteria-free filtrate plus ascorbic acid. A control series also was made on autoclaved and bacteria-free filtrate

without added ascorbic acid. In the former, ascorbic acid was approximately twice as high as in untreated samples. The bacteria-free filtrate contained no more ascorbic acid than untreated rumen juice.

The results reveal that the added ascorbic acid was lost in untreated and starch-treated rumen juice in 30 minutes, with the latter showing less loss at 15 minutes than the former. The rate of loss in autoclaved and filtered material was considerably slower. Although precautions were taken to prevent bacterial contamination, some undoubtedly occurred in each case. However, the rate of loss in these samples would indicate that factors other than rumen flora alone may be involved in the loss of ascorbic acid in rumen juice.

DISCUSSION

In vivo. Samples of ingesta from the various fistulae taken during the control series showed pH values of rumen juice ranging from 5.72 to 6.56; the range of all pH determinations during all trials was 5.72 to 7.46. This range of values is essentially that reported by Dukes (1), Kick *et al.* (5), Monroe and Perkins (7), and Smith (9). The pH values of colon and cecum contents generally were between 7.00 and 8.00 except when large quantities of ascorbic acid were present. The pH of jejunal contents also was alkaline, showing a range of 7.22 to 8.16.

Ascorbic acid was recovered from samples of rumen juice for 2 hours following administration of 16 g. of the vitamin. This is essentially in agreement with Vavich *et al.* (11), although these authors administered as much as 150 g. of ascorbic acid at one time. Ascorbic acid was recovered from the colon 4 hours after treatment and for as long as 24 hours in cecal contents.

The administration of ascorbic acid subcutaneously and intravenously or of chlorobutanol into various levels of the digestive tract had no noticeable effect on the ascorbic acid content of rumen or colon contents.

There was evidence that ascorbic acid was eliminated in the feces in increased amounts following administration of ascorbic acid into the rumen, colon, and cecum, with the latter showing considerably more elimination at 8 hours after treatment.

Fecal elimination of ascorbic acid did not increase following the addition of chlorobutanol to the rumen, colon, and cecum. Neither was there any evidence that the subcutaneous or intravenous administration of ascorbic acid resulted in any increased fecal elimination. During these trials the range was from 0 to 2.38 mg. per cent ascorbic acid. The amount of ascorbic acid in the feces of the steers was several times less than that reported for dairy cows by Wallis (12). However, the age, type of animal, difference in ration, environment, and the presence of fistulae in the steers prevent any detailed comparisons between the two experiments.

In vitro. Whitnah and Riddell (13), Knight *et al.* (6), and Vavich *et al.* (11) observed rapid loss of ascorbic acid from rumen contents. Young and

James (14) and Young and Rettger (15) observed that certain organisms of the intestinal flora utilized or aided in the loss of ascorbic acid.

Incubation of digestive tract contents containing ascorbic acid added *in vivo* revealed that most of the vitamin was lost from rumen juice after 11 hours of incubation. Appreciable quantities remained in colon contents after 24 hours of incubation. Loss from small intestinal contents occurred at approximately the same rate as was observed in the case of colon contents.

The addition of ascorbic acid to rumen juice at the rate of 0.25 and 0.5 g. per 100 c.c. and incubation at 37° C., indicated approximately 250 mg. of ascorbic acid were lost from each sample during the first 24 hours and, in the case of the higher concentration, the remainder was lost during the next 20 hours. When 0.5 g. of ascorbic acid was added to 100 c.c. of colon contents and incubated at 37° C., the rate of loss approximated that observed for rumen juice. However, the samples containing only half as much ascorbic acid lost the vitamin only one-third as fast. The rate of loss of ascorbic acid added to rumen juice was considerably less than was observed by Vavich *et al.* (11). However, these authors incubated their samples at 39.5° C. and determined only reduced ascorbic acid.

Though the concentration of ascorbic acid was approximately twice as high in feces and in the colon and cecal contents as compared to rumen juice or juice from the jejunum, this does not necessarily mean that any lower bowel synthesis of ascorbic acid has taken place. The increase in dry matter in the lower bowel easily could account for the differences noted. Furthermore, to the authors' knowledge, no information is available on how much ascorbic acid might be found in the living microflora of the digestive tract. According to Hastings (3), the amount of microflora on a dry-matter basis is approximately the same for rumen and fecal material. Thus differences in ascorbic acid could be due to the concentration of microflora. The other possibility is the liberation of the vitamin from undigested portions of grain and hay as they proceed along the digestive tract. Getting more of this solid material in the sample easily could account for the differences observed.

Further studies on rumen juice revealed that ascorbic acid was lost quite slowly from a bacteria-free filtrate and autoclaved samples as compared to samples not treated in this manner. This essentially is in agreement with Vavich *et al.* (11).

The addition of corn starch to rumen juice of a cow on pasture and receiving no grain showed a slight "sparing" effect on ascorbic acid. Adding thiourea or chlorobutanol or both to rumen juice had no effect on the rate of loss of ascorbic acid. These studies indicate that both microbiological and chemical factors are involved in the loss of ascorbic acid from rumen juice. This loss cannot be attributed solely to bacteria or enzymes.

SUMMARY

1. The ascorbic acid content of rumen juice, intestinal juice, colon and cecal contents, and feces was determined during ascorbic acid absorption studies with steers.

2. Ascorbic acid was recovered from rumen juice for 2 hours following rumen administration of 16 g. of ascorbic acid. The vitamin was recovered for 4 and 24 hours, respectively, from colon and cecal contents after placing 4 g. of the vitamin directly into these regions.

3. The average ascorbic acid content of feces, cecal, and colon contents was approximately twice as high as jejunal or rumen juice during periods when ascorbic acid was not administered.

4. Placing chlorobutanol in the digestive tract or giving ascorbic acid subcutaneously or intravenously had no apparent effect on the level of ascorbic acid in digestive tract materials.

5. Some fecal elimination of ascorbic acid was evident following administration of ascorbic acid into the digestive tract.

6. *In vivo* and *in vitro* studies on rumen and jejunal juice and on colon contents indicated ascorbic acid was quite labile in these media, even though considerable quantities were recovered *in vivo* several hours after treatment. *In vitro* samples incubated at 37° C. lost ascorbic acid less rapidly.

7. Some ascorbic acid administered into the rumen, colon, and cecum was eliminated in the feces, with the latter showing the greatest elimination.

8. Autoclaving or preparing bacteria-free filtrates of rumen juice greatly retarded loss of ascorbic acid as compared to untreated rumen juice.

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OBSERVATIONS ON VITAMIN A DEFICIENCY IN YOUNG DAIRY BULLS[1]

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Vitamin A deficiency results in the cessation of spermatogenesis and atrophy of the testes in laboratory animals (11, 14), and recent studies (2, 3, 4, 5, 9, 10, 12, 13) have shown vitamin A deficiency to have a similar effect on the testes of the bull. It also has been reported (2, 3, 5, 6, 9, 10) that damage to germinal tissue of the testis due to vitamin A deficiency is reparable. The development of pituitary cysts has been attributed to vitamin A deficiency (5, 10, 12); this condition appears to be irreparable by vitamin A therapy. The common clinical symptoms of vitamin A deficiency are apt to occur prior to any recognizable reduction of fertility of bulls (5, 6, 9), especially when the deficiency develops after puberty.

The purpose of this study was to determine the effect of suboptimum intake of vitamin A upon reproduction in the male bovine and to determine whether or not prepubertal vitamin A deficiency results in permanent impairment of the reproductive processes which cannot be overcome by vitamin A therapy.

PROCEDURE

Two trials were conducted between August, 1944, and December, 1946. The first trial consisted of two groups of five bulls each, and the second trial of three groups of four bulls each. The levels of vitamin A intake and age when placed on the experiment are shown in table 1. The calves were handled in a routine manner prior to being placed on experiment. During the experiment all bulls were fed beet pulp and a grain mixture containing 100 lb. ground white corn, 85 lb. ground oats, 45 lb. wheat bran, 30 lb. linseed oil meal, 45 lb. soybean oil meal, 1.5 lb. iodized salt, 1.5 lb. bone meal, 1.5 lb. pulverized limestone, and 0.5 lb. irradiated yeast. Vitamin A (concentrated liver oil) was supplied daily as outlined in the table.

The various groups were kept in separate pens and exercising lots; each bull was tied during feeding. Semen collection was made with the artificial vagina, using estrus females. Blood plasma vitamin A determinations were made at bimonthly intervals on bulls in trial II from February to December, 1946, using the method of Kimble (7); blood carotene, ascorbic acid, and hemoglobin also were determined, but these data will not be presented in

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TABLE 1

Daily vitamin A intake and ages of animals on experiment

Trial and group	Herd no.	Breed	Initial age	Interval on expt.	Vitamin A		Remarks
					Date	Intake	
			(*days*)	(*days*)		(*I.U. daily*)	
I—Control	B548	Hol.	105	501	7/9/44	100,000	Sold 8/10/45
I—Control	B550	Hol.	94	505	7/9/44	100,000	Slaughtered 8/24/45
I—Control	B558	Hol.	63	436	8/23/44	100,000	Sold 8/31/45
I—Control	P111	Jer.	44	432	7/9/44	100,000	Slaughtered 8/1/45
I—Control	P112	Jer.	44	60	7/9/44	100,000	Died—cause unknown 9/7/44
I—Low	B549	Hol.	103	698	7/9/44	2,000	
					12/14/44	4,000	Testicular biopsy 9/19/45
					3/9/45	6,000	Slaughtered 2/26/46
I—Low	B555	Hol.	48	643	7/9/44	2,000	
					12/14/44	4,000	
					3/9/44	6,000	
					9/19/44	100,000	Testicular biopsy 9/19/45; slaughtered 2/26/46
I—Low	B557	Hol.	40	616	7/28/44	Same as B555	Same as B555
I—Low	B554	Guer.	56	446	7/9/44	2,000	
					12/14/44	4,000	
					3/9/44	6,000	Slaughtered 8/3/45
I—Low	B553	Guer.	58	202	7/9/44	2,000	
					12/14/44	4,000	Died: pneumonia 12/20/44

TABLE 1—(*Continued*)

Trial and group	Herd no.	Breed	Initial age	Interval on expt.	Vitamin A		Remarks
					Date	Intake	
II—Control	B564	Hol.	44	532	8/1/45	100,000	Slaughtered 12/12/46
II—Control	B563	Guer.	75	573	8/1/45	100,000	Slaughtered 12/12/46
II—Control	B576	Hol.	44	28	2/19/46	100,000	Died: rumenitis 3/19/46
II—Control	P120	Jer.	49	488	9/29/45	100,000	Slaughtered 12/12/46
II—Medium	B565	Hol.	42	525	8/16/45 3/5/46	6,000 16/lb.	Slaughtered 12/11/46
II—Medium	B569	Hol.	45	474	10/9/45 5/12/46	6,000 16/lb.	Slaughtered 12/11/46
II—Medium	B575	Hol.	50	347	2/19/46 6/1/46	6,000 16/lb.	Slaughtered 12/11/46
II—Medium	P118	Jer.	50	548	8/1/45 4/18/46	6,000 16/lb.	Slaughtered 12/11/46
II—Low	B566	Hol.	42	519	8/16/45 3/5/46	4,000 8/lb.	Slaughtered 12/6/46
II—Low	B570	Hol.	42	454	10/20/45 4/18/46	4,000 8/lb.	Slaughtered 12/6/46
II—Low	B573	Hol.	44	383	1/8/46 7/16/46	4,000 8/lb.	Slaughtered 12/12/46
II—Low	P119	Jer.	42	525	8/16/45 4/18/46	4,000 8/lb.	Slaughtered 12/12/46

detail. Growth rate was measured and observations made for the clinical symptoms of vitamin A deficiency.

RESULTS

Trial I. As shown in table 1, two groups of five bulls each were started on experiment during July and August, 1944. On September 7, 1944, a Jersey bull (P112) in the control group died (cause undetermined), and on December 20, 1944, a Guernsey bull (B553) in the low vitamin A group died of pneumonia. This left three Holsteins and one Guernsey in the low group, and three Holsteins and one Jersey in the control group. As shown in figure 1, the high group grew satisfactorily throughout the experiment.

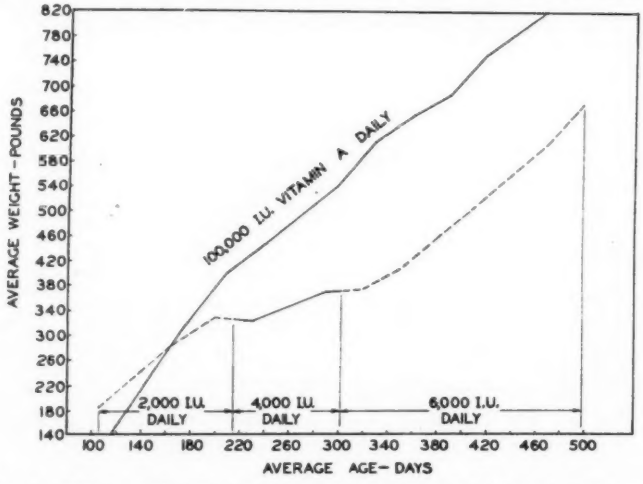

FIG. 1. Effect of vitamin A intake on rate of growth in dairy bull calves.

In December, 1944, bulls in the low group receiving 2,000 I.U. vitamin A daily began to lose weight, showing incoordination and reduced feed intake. By December 14, three of the four bulls in the low group were blind, and the vitamin A intake of all bulls in the group was raised to 4,000 I.U. daily. The bulls slowly recovered and gained at a low rate. The bull (B557) which had not gone blind while on 2,000 I.U. of vitamin A daily showed distinct blindness on February 23, 1945, while on 4,000 I.U. On March 9, 1945, all bulls were raised to 6,000 I.U. vitamin A daily (approximately 14 I.U. per lb. of weight). As body weight increased, the daily vitamin A intake decreased, until by September, 1945, it was about 9 I.U. per lb.

Satisfactory semen was produced by the bulls in the control group at 16 months of age, but only slight spermatogenesis was noted in bulls on the low-level intake. Two bulls from the control group (B550, P111) and one bull

from the low group (B554) were slaughtered and autopsied during August, 1945. Histological sections were made of the vital organs, endocrine glands, and reproductive tracts. No gross pathology was evident in the bulls from the control group and only blindness in the bull from the low group. Histological examination of the testes revealed a moderate rate of spermatogenesis in all three bulls.

Testicular biopsies (2) were made on the three remaining Holstein bulls (B549, B555, B557) on September 19, 1945. Histological sections of the testes revealed retarded proliferation of the germinal epithelium, indicating delayed sexual maturity. Two of these bulls (B555, B557) then were raised to 100,000 I.U. of vitamin A daily while the other (B549) remained on 6,000 I.U. Although these bulls were blind, they gradually became accustomed to mounting cows in heat. Twelve samples of semen were collected from each bull during January and February, 1946. In only one sample (B549) was initial motility rated above 60 per cent. The concentration of sperm generally was below 500,000 per $mm.^3$ and survival time at 5° C. usually was less than 24 hours. The sex desire of these bulls was quite good. B549 (6,000 I.U. daily) consistently produced the best quality of semen, while the semen of B557 was of very inferior quality. These bulls were slaughtered and autopsied February 26, 1946. All three had cystic pituitaries. B555 had the largest cyst (approximately 2.5 cm. in diameter) and also showed gross kidney damage; histological examination of the kidneys revealed extensive albuminous degeneration of the convoluted tubules, leucocytic infiltration, and glomerular damage. The kidneys of B549 and B557 showed only very slight albuminous degeneration. The testes of these bulls showed a moderate rate of spermatogenesis, with germinal elements arranged fairly densely. The epididymis contained moderate numbers of stored sperm.

Trial II. This trial was started in August, 1945. The animals were placed on experiment as they became available. The details are shown in table 1. Bulls in the medium group received 6,000 I.U. vitamin daily until they reached 300 lb. in weight. The vitamin A intake then was adjusted to 16 I.U. per lb. daily. The low group received 4,000 I.U. daily until they reached 300 lb. (B566 until 400 lb.); intake then was adjusted to 8 I.U. per lb. daily. All bulls were weighed on 3 consecutive days each month and at such other times as necessary to regulate the vitamin A intake. These vitamin levels were selected because it was believed that 8 I.U. per lb. would be marginal and 16 I.U. per lb. would be adequate. The average intake of vitamin A per pound of body weight and rate of gain for each weight period for each bull used in this trial are shown in table 2. None of the bulls with the exception of B575 in the medium group grew as rapidly as the control bulls in trial I (fig. 1). This failure to duplicate growth rate was due partially to not attempting to feed for maximum growth, especially prior to March, 1946. The controls in this experiment (trial II) showed unsatisfactory

TABLE 2

Growth rate and daily vitamin A intake

Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)
B566 (Low group)				
Birth	0	92	.....	.....
8–16–45	42	125	.....	0.79
9–20–45	35	168	27.3	1.28
10–24–45	34	195	22.0	0.79
12–1–45	38	263	17.5	1.79
1–1–46	31	301	14.2	1.23
2–1–46	31	345	12.4	1.42
3–5–46	32	398	10.8	1.34
3–29–46	24	410	8.4	0.50
4–30–46	33	463	7.8	1.61
5–31–46	31	503	7.9	1.29
6–30–46	30	543	8.1	1.33
7–31–46	31	560	8.2	0.74
8–31–46	31	590	8.0	0.97
10–1–46	31	622	8.1	1.03
10–31–46	30	667	8.0	1.50
12–5–46	35	700	7.9	0.94
B570 (Low group)				
Birth	0	86	.....	.....
10–20–45	42	100	.....	0.33
10–30–45	10	105	39.0	0.50
12–1–45	32	132	33.8	0.84
1–1–46	31	170	26.5	1.23
2–1–46	31	222	20.4	1.68
3–1–46	28	263	16.5	1.46
4–18–46	48	300	14.2	0.77
4–30–46	12	312	8.5	1.00
5–31–46	31	357	8.0	1.45
6–30–46	30	396	8.0	1.30
7–31–46	31	443	8.1	1.52
8–31–46	31	495	8.1	1.68
10–1–46	31	545	8.1	1.61
10–31–46	30	575	8.2	1.00
12–5–46	35	566	8.1	−0.26

Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)
B565 (Medium group)				
Birth	0	87	.....	.....
8–16–45	42	101	.....	0.33
9–20–45	35	135	50.8	0.97
10–24–45	34	172	39.1	1.09
12–1–45	38	224	30.3	1.37
1–1–46	31	231	26.4	0.23
2–1–46	31	268	24.0	1.19
3–5–46	32	298	21.2	0.94
3–29–46	22	311	17.1	0.59
4–30–46	32	353	15.7	1.31
5–31–46	31	392	16.1	1.26
6–30–46	30	415	16.9	0.77
7–31–46	31	468	15.4	1.71
8–31–46	31	519	16.3	1.65
10–1–46	31	595	15.9	2.45
10–31–46	30	648	16.1	1.77
12–11–46	41	711	16.1	1.54
B569 (Medium group)				
Birth	0	95	.....	.....
10–9–45	45	97	.....	0.04
11–1–45	23	118	55.8	0.91
12–1–45	30	142	46.2	0.80
1–1–46	31	165	39.1	0.74
2–1–46	31	204	32.5	1.26
3–1–46	28	230	27.6	0.93
3–29–46	28	252	24.9	0.79
5–12–46	44	301	21.7	1.11
5–31–46	19	321	16.7	1.05
6–30–46	30	348	15.5	0.90
7–31–46	31	377	16.6	0.94
8–31–46	31	431	16.0	1.74
10–1–46	31	475	16.0	1.42
10–31–46	30	504	16.1	0.97
12–11–46	41	523	16.4	0.46

Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)
B564 (High group)				
Birth	0	84	.....	.....
8–1–45	44	98	.....	0.32
9–2–45	32	134	862.0	1.12
10–13–45	41	193	611.6	1.44
11–1–45	19	215	490.2	1.16
12–1–45	30	242	437.6	0.90
1–1–46	31	266	393.7	0.77
2–1–46	31	292	358.4	0.84
3–1–46	28	311	331.7	0.68
3–29–46	28	337	308.6	0.93
4–30–46	32	393	274.0	1.75
5–31–46	31	402	251.6	0.29
6–30–46	30	449	235.0	1.57
7–31–46	31	500	210.7	1.65
8–31–46	31	529	194.4	0.94
10–1–46	31	559	183.8	0.97
10–31–46	30	625	168.9	2.20
12–11–46	41	673	154.1	1.17
B563 (High group)				
Birth	0	61	.....	.....
8–1–45	75	122	.....	0.81
9–2–45	32	147	743.5	0.78
10–13–45	41	201	574.7	1.32
11–1–45	19	219	476.2	0.95
12–1–45	30	238	437.6	0.63
1–1–46	31	257	404.0	0.61
2–1–46	31	270	379.5	0.42
3–1–46	28	296	353.4	0.93
3–29–46	28	326	321.5	1.07
4–30–46	32	355	293.7	0.91
5–31–46	31	404	263.5	1.58
6–30–46	30	441	236.7	1.23
7–31–46	31	486	215.7	1.45
8–31–46	31	532	196.5	1.48
10–1–46	31	588	178.6	1.81
10–31–46	30	635	163.5	1.57
12–11–46	41	583	164.2	−1.27

TABLE 2—(*Continued*)

Growth rate and daily vitamin A intake

Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)	Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)	Date	Interval (days)	Wt. (lb.)	Av. vitamin level (I.U./lb.)	Av. daily gain (lb.)
B573 (Low group)					B575 (Medium group)									
Birth	0	96	.....	.....	Birth	0	99	.....	.....					
1–8–46	44	111	.....	0.34	2–19–46	50	156	.....	1.12					
3–1–46	52	154	30.2	0.83	3–29–46	38	187	35.0	0.82					
3–29–46	28	177	24.2	0.82	4–30–46	32	243	27.9	1.75					
4–30–46	32	210	20.7	1.03	5–31–46	31	302	22.0	1.90					
5–31–46	31	251	17.4	1.32	6–30–46	30	353	15.9	1.70					
6–30–46	30	285	14.9	1.13	7–31–46	31	405	15.8	1.68					
7–16–46	16	302	13.6	1.06	8–31–46	31	477	15.4	2.32					
7–31–46	15	327	8.3	1.67	10–1–46	31	547	15.7	2.26					
8–31–46	31	379	7.8	1.68	10–31–46	30	605	16.0	1.93					
10–1–46	31	441	8.0	2.00	12–11–46	41	682	15.8	1.88					
10–31–46	30	473	8.3	1.07										
12–11–46	41	531	7.7	1.41										
P119 (Low group)					P118 (Medium group)					P120 (High group)				
Birth	0	51	.....	.....	Birth	0	54	.....	.....	Birth	0	62	.....	.....
8–16–45	42	66	.....	0.36	8–1–45	50	73	.....	0.38	9–29–45	49	73	.....	0.22
9–20–45	35	96	49.4	0.86	9–2–45	32	104	67.8	0.97	11–1–45	33	100	1156.0	0.82
10–24–45	34	121	36.9	0.74	10–13–45	41	153	46.7	1.20	12–1–45	30	130	869.6	1.00
12–1–45	38	156	28.9	0.92	12–1–45	49	204	33.6	1.04	1–1–46	31	151	711.7	0.68
1–1–46	28	193	22.3	1.19	1–1–46	31	216	28.6	0.39	2–1–46	31	173	617.3	0.71
2–1–46	31	235	18.7	1.35	2–1–46	31	242	26.2	0.84	3–1–46	28	188	554.0	0.54
3–1–46	28	266	16.0	1.11	3–1–46	28	246	24.6	0.14	3–29–46	28	203	511.5	0.54
4–18–46	48	309	13.9	0.90	3–29–46	28	267	23.4	0.75	4–30–46	32	209	485.4	0.19
4–30–46	12	324	8.2	1.25	4–18–46	20	301	21.1	1.70	5–31–46	31	249	436.7	1.29
5–31–46	31	354	7.9	0.97	4–30–46	12	316	16.9	1.25	6–30–46	30	296	367.0	1.57
6–30–46	30	367	8.3	0.43	5–31–46	31	364	15.8	1.55	7–31–46	31	343	313.0	1.52
7–31–46	31	401	7.8	1.10	6–30–46	30	394	15.8	1.00	8–31–46	31	404	267.7	1.97
8–31–46	31	448	8.0	1.52	7–31–46	31	449	16.1	1.77	10–1–46	31	470	228.8	2.13
10–1–46	31	507	8.1	1.90	8–31–46	31	507	15.9	1.87	10–31–46	30	520	202.0	1.67
10–31–46	30	547	8.0	1.33	10–1–46	31	557	16.2	1.61	12–11–46	41	593	179.7	1.78
12–11–46	41	614	7.9	1.63	10–31–46	30	594	16.0	1.23					
					12–11–46	41	632	16.3	0.93					

growth rates and were off feed more times than the bulls in the other two groups (table 3). Between-group comparisons are not possible since the control group contained a Holstein, Guernsey, and Jersey, whereas the other two groups were composed of three Holsteins and one Jersey. Bulls in both the low and medium groups showed night blindness during the experiment (table 3); all except P119 (low group) had convulsions at one time or another, with B566 and B570 having the most serious and most frequent attacks; three bulls, B566 and B570 (low group), and B118 (medium group) went blind. All animals in the low and medium groups showed considerable papillary edema, and retinal hemorrhage was observed in three cases (P118, B566, B570). No other clinical symptoms of vitamin A deficiency were observed. The animals remained on feed and grew as well as the controls (table 2). Their hair coats had a fine, sleek appearance and the hides were pliable. No swelling was observed in the feet or legs.

Beginning in February, 1946, blood analyses for vitamin A, carotene, hemoglobin, and ascorbic acid were made bimonthly. Blood plasma carotene was of no consequence since these animals were receiving vitamin A. The hemoglobin increased slowly with increasing age, with no observable differences between groups, and whole blood and blood plasma ascorbic acid were not appreciably different between the groups. The results of blood plasma vitamin A analyses are shown in table 4. Bulls in the medium and low groups showed essentially the same low concentration of vitamin A in the blood plasma, which is further indication of suboptimum vitamin A intake. The controls were considerably higher. Boyer *et al.* (1) reported that blood plasma vitamin A levels of 10 γ or more per 100 c.c. are necessary for adequate vitamin A nutrition of the growing calf and that plasma values of 7–8 γ are borderline.

Semen collections were attempted on all bulls beginning at 13 months of age. Ten or more attempts were made with each animal. Although each bull was observed riding others, only the Guernsey control (B563) produced semen of high quality. Very few sperm were observed in the ejaculates from B564 (control) and B570 (low), and these showed only slight motility. The other bulls that ejaculated (B573, B565, B575) produced no sperm. The ages at first ejaculation are shown in table 3. The bulls from which no ejaculates were obtained showed no interest in estrus cows at collection time.

Since bulls in the low and medium groups all showed various stages of vitamin A deficiency during November, 1946, all bulls were slaughtered during the following month. The vital organs were weighed and histological sections made. Organ weights and percentages of body weight are shown in table 5. The results of histological examination of the testes are shown in table 6. The organs and glands not otherwise described appeared histologically normal.

No pituitary cysts developed in the five control bulls which were autopsied

TABLE 3

Observations of bulls in trial II

Group	Bull	Days on experiment when observed					Age at 1st ejaculation	Remarks
		Off feed	Scouring	Convulsions	Night blindness	Blindness		
		(*days*)	(*days*)	(*days*)	(*days*)	(*days*)	(*days*)	
Low	B566	.....	.....	392	352	430	.....	
	B570	359, 375, 401	.....	329	263	390	449	Slight pneumonia 11/15/45
	B573	.....	.....	318	248	.....	377	
	P119	326	.....	.....	454	.....	.....	
Medium	B565	.....	.....	457	352	.....	432	
	B569	370, 386	310	400	293	.....	.....	Scoured considerably after 8/46
			330	.....	.....	.....	.....	
	B575	.....	.....	294	207	.....	340	
	P118	412	.....	457	352	388	.....	Hoof rot 7/10/46
Control	B564	446	.....	.....	.....	.....	495	
	B563	249, 443, 454	.....	.....	.....	.....	407	Pneumonia 11/3/46
	P120	182, 206, 274	.....	.....	.....	.....	.....	Hoof rot 11/19/46
		382	.....	.....	.....	.....	.....	

TABLE 4

Micrograms of vitamin A per ml. of blood plasma

Date* (1946)	Low group				Medium group				High group		
	B566	B570	B573	P119	B565	B569	B575	P118	B564	B563	P120
Mar. 8	0.23	0.18	0.18	0.16	0.14	0.14	0.17	0.16	0.27	0.32	0.35
April 18	0.16	0.13	0.10	0.13	0.13	0.13	0.19	0.16	0.23	0.34	0.30
May 31	0.13	0.12	0.11	0.22	0.17	0.13	0.19	0.18	0.39	0.38	0.37
July 11	0.12	0.20	0.17	0.23	0.19	0.11	0.12	0.14	0.32	0.36	0.41
Aug. 24	0.12	0.11	0.12	0.15	0.13	0.07	0.12	0.14	0.39	0.45	0.52
Oct. 4	0.10	0.11	0.12	0.23	0.20	0.15	0.17	0.22	0.43	0.57	0.50
Nov. 14	0.09	0.08	0.10	0.12	0.11	0.06	0.07	0.10	0.31	0.33	0.40
Dec. 12†	0.05	0.05	0.04	0.10	0.06	0.04	0.04	0.10	0.31	0.30	0.46

* Av. of three determinations made on the date, 2 weeks prior, and 2 weeks after.
† Av. of three consecutive daily samples taken prior to slaughter.

TABLE 5

Organ weights and percentage of body weight

Organ	Wt.	% body wt.	Wt.	% body wt.	Wt.	% body wt.	Wt.	% body wt.
Low group	B566		B570		B573		P119	
Testes (g.)	410.9	0.129	350.5	0.137	186.0	0.077	278.1	0.100
Epid. (g.)	40.9	0.013	26.7	0.010	15.6	0.006	31.3	0.011
S. vesc. (g.)	40.4	0.013	34.9	0.014	38.0	0.016	43.7	0.016
Cowpers (g.)	4.1	0.001	3.1	0.001	3.1	0.001	4.4	0.002
Pit. (g.)*	1.6	0.0005	2.0	0.0008	1.5	0.0006	1.5	0.0006
Thyroid (g.)	22.2	0.007	23.8	0.009	16.2	0.007	13.2	0.005
Adr. (g.)	16.3	0.005	6.8	0.003	11.0	0.005	12.2	0.004
Medium group	B565		B569		B575		P118	
Testes (g.)	240.3	0.074	201.8	0.085	248.0	0.082	414.3	0.144
Epid. (g.)	46.6	0.014	18.2	0.008	20.6	0.007	32.6	0.011
S. vesc. (g.)	75.8	0.024	44.9	0.019	62.9	0.020	40.5	0.014
Cowpers (g.)	4.3	0.001	3.5	0.002	4.7	0.002	4.8	0.002
Pit. (g.)*	2.2	0.0007	1.6	0.0007	1.7	0.0005	1.9	0.0007
Thyroid (g.)	23.3	0.007	18.4	0.008	35.7	0.012	19.9	0.007
Adr. (g.)	15.8	0.005	10.9	0.005	11.4	0.004	13.4	0.005
Control group	B564		B563				P120	
Testes (g.)	264.9	0.087	344.4	0.130			360.6	0.134
Epid. (g.)	36.3	0.012	37.8	0.014			24.6	0.009
S. vesc. (g.)	48.6	0.016	59.0	0.022			39.5	0.015
Cowpers (g.)	3.6	0.001	4.5	0.002			5.0	0.002
Pit. (g.)*	1.7	0.0006	1.9	0.0007			1.8	0.0007
Thyroid (g.)	23.3	0.008	22.2	0.008			15.4	0.006
Adr. (g.)	11.9	0.004	19.0	0.007			14.4	0.005

* Whole pituitary minus cyst.

in trials I and II. Edema and vacuolation of the basophiles were observed in B550 and P120. Seven of the nine bulls receiving the low level of vitamin A developed pituitary cysts ranging in diameter from 0.5 to 3 cm. The pituitary histology of the bulls without cysts (B553 and B554) was relatively normal. The remainder of the pituitaries of this group were characterized

TABLE 6

Trial II—histological observations on testes and epididymides

Group	Bull no.	Testes	Epididymides
Low	B566	Low rate of spermatogenesis. Some tubules contained only spermatogonia.	Left: few stored sperm, some leucocytic infiltration; right: large number of stored sperm.
Low	B570	Very low rate of spermatogenesis. Primary and secondary spermatocytes only in most tubules.	Moderate numbers of sperm.
Low	B573	No sperm. Most tubules contained only spermatogonia and primary spermatocytes.	No sperm.
Low	P119	Low rate of spermatogenesis. Some tubules with no lumina and containing only spermatogonia and a few primary spermatocytes.	Abundant stored sperm.
Medium	B565	Very low rate of spermatogenesis. Some tubules with no lumina and containing only spermatogonia and a few primary spermatocytes.	No sperm.
Medium	B569	An occasional sperm. Most tubules had primary and secondary spermatocytes only.	Epithelial cells in various stages of mitosis and a few sperm heads.
Medium	B575	No sperm. Most tubules contained spermatogonia and secondary spermatocytes, and a few spermatids.	No sperm. Few epithelial cells.
Medium	P118	Low rate of spermatogenesis. Some tubules with primary spermatocytes and spermatogonia only.	Large numbers of sperm.
Control	B564	Low rate of spermatogenesis.	Left: numerous areas with leucocytic infiltration, moderate sperm storage; right: abundant sperm.
Control	B563	Moderate spermatogenesis.	Abundant sperm.
Control	P120	Moderate spermatogenesis.	Abundant sperm.

by a loose arrangement of the anterior pituitary cells and vacuolation of the basophiles, and edema was observed in five of the glands. All of the four bulls in the medium vitamin A group in trial II developed pituitary cysts. Two of the bulls (B565 and B569) showed some edema, vacuolation of the basophiles, and a loose arrangement of the pituitary elements.

No consistent differences occurred between groups in the histology of the

adrenal cortex but some variation from normal appeared in the adrenal medulla. In one of the control bulls (B550) slight necrobiosis of the medulla was observed, and in a second control (B564) there was moderate lymphocytic infiltration of the medulla. In five of the nine bulls on low vitamin A intake early necrobiosis or necrosis of the medullary cells was observed and in one (B555) several large areas of polymorphonuclear leucocytes extended from the capsule through the medullary portion. In the medium vitamin A group early necrobiosis was observed in B565 and B569, the bulls with the largest pituitary cysts in the group. One area of the cortex was infiltrated with polymorphonuclear leucocytes in P118.

DISCUSSION

There is general agreement among investigators that vitamin A deficiency, if prolonged, results in the degeneration of the germinal epithelium of the testes in sexually mature bulls and that lowered fertility or sterility results. It is likewise agreed, that in sexually mature bulls, relatively normal spermatogenesis can be restored by vitamin A therapy (2, 3, 4, 5, 9, 12). The effects of prepubertal vitamin A deficiency upon sexual development and subsequent fertility or reproductive capacity are not completely understood. Following the reports of Madsen *et al.* (10) that prenatal or postnatal vitamin A deficiency in cattle results in the development of pituitary cysts which are not reparable by vitamin A therapy, it was hypothesized that suboptimal vitamin A intake prior to puberty might be a cause of lowered fertility in mature bulls.

It originally was intended that the level of vitamin A intake in the Purdue experiment would prevent clinical vitamin A deficiency and that the results of suboptimal vitamin A could be measured for the most part in terms of semen quality. As reported above, the results were not wholly as expected. In the first experiment, three Holstein bulls on low vitamin A intake became blind by 6 months of age. The development of blindness resulted in continual difficulty in making semen collections, and because of this complication, it is impossible to separate the physiological and psychological effects of vitamin A deficiency on reproductive capacity. However, the microscopic study of testicular biopsies made on the three bulls at 14 months of age showed that spermatogenesis was retarded. Two of the animals received 100,000 I.U. of vitamin A daily beginning at the fourteenth month and one was held at 6,000 I.U. Although blindness persisted, all of the bulls eventually ejaculated. None of the semen samples could be classified as of excellent quality, although large numbers of sperm were produced. Examination of the testes at autopsy revealed that the rate of spermatogenesis was suboptimum in terms of bulls of known high fertility. Evidences of pituitary and adrenal damage were observed. Although the authors are not specialists in pituitary or adrenal cytology, certain histo-pathological changes were noted.

Pituitary cysts were present in the three bulls. The cells of the anterior pituitaries were loosely arranged; there were evidences of edema, and the basophiles showed more vacuolation than in normal bulls. Clear-cut castration cells were not observed. Although vitamin A therapy was carried out for 5 months, spermatogenesis was not restored to an entirely satisfactory level and histological evidences of vitamin A deficiency persisted. These results are in essential agreement with those of Madsen *et al.* (10) and Hodgson *et al.* (5). It would appear, however, that acute vitamin A deficiency in sexually immature animals may have effects which persist long after the correction of the deficiency.

The second experiment was planned with the intention of studying both vitamin A deficiency and subclinical deficiency in prepubertal bulls. Vitamin A levels of 4,000 and 6,000 I.U. per day until the bulls reached 300 lb., and adjustment at that time to 8 and 16 I.U. of vitamin A per lb. body weight, were maintained. These levels are equivalent to 17.6 and 35.3 I.U. per kg. of body weight and are approximately one-half and slightly above the minimum requirements (32 I.U. per kg.) for calves as reported by Lewis and Wilson (8). As previously pointed out, neither the control nor experimental bulls in trial II grew as rapidly as the controls in trial I. This difference apparently is the result of a lower daily feed intake than was maintained in the first experiment. All of the bulls in the low and medium vitamin A groups developed night blindness and all except one (P119—low group) had convulsions during the trial. Papillary edema and retinal hemorrhage were observed in three bulls, and blindness developed in three cases. However, other clinical evidences of vitamin A deficiency were not observed. Growth rate of the bulls in the low and medium groups was essentially similar to that of the controls receiving 100,000 I.U. of vitamin A daily, and the hides were sleek and pliable. Normal sexual maturity, as measured by sexual drive and semen quantity and quality, was retarded in the low and medium groups.

Histological studies of the entire reproductive tract and the vital organs were made. The rate of spermatogenesis in eight of the experimental and one of the control bulls was low and definitely suboptimal. Sperm production was reduced.

Although Hodgson *et al.* (5) demonstrated fertility in the semen of vitamin A-deficient bulls, they found that semen quality as measured by the usual standards was low. It is the opinion of the authors that although bulls receiving deficient or slightly deficient rations may not be sterile, fertility is affected and reproductive capacity definitely is limited. The reduction of sexual drive and failure to ejaculate are serious reproductive disturbances. The ability of males with these characteristics to impregnate an occasional female is an indication of fertility but is no measure of normal reproductive capacity.

The development of recognizable pituitary and adrenal changes in the

bulls receiving 8 and 16 I.U. of vitamin A suggests that these intakes do not permit complete physiological development, even though growth may be relatively satisfactory.

The results which have been obtained in these and other studies on the vitamin A requirements of growing bulls leave much to be desired. Variations between bulls of the same and different breeds, failure to adjust vitamin A intake to body weight from birth, and the failure to conduct blood vitamin A studies beginning at birth all have contributed to the difficulty of reaching entirely satisfactory conclusions in this study. The interference of blindness with normal behavior may account for some of the observations on the apparent lack of sexual drive, but there seems to be sufficient evidence to suggest that suboptimal vitamin A intake retards male hormone production as well as spermatogenesis.

SUMMARY

1. The effects of varying levels of vitamin A on the reproductive development of 13 Holstein, 4 Jersey, and 3 Guernsey bulls were studied.

2. Complete blindness developed in bulls receiving 2,000 I.U. of vitamin A daily. Following supplementation of the ration with 4,000 and 6,000 I.U. of vitamin A daily, very slight spermatogenesis was observed; only moderate sperm production was found following 5 months of therapy with 100,000 I.U. of vitamin A daily.

3. Bulls which received 4,000 and 6,000 I.U. daily of vitamin A until 300 lb. in weight, and then 8 and 16 I.U. daily per lb. of body weight, exhibited convulsions, showed evidence of night blindness, and three became totally blind. Sexual maturity was delayed, sex drive inhibited, and spermatogenesis limited. Cystic pituitary glands were observed in all bulls except the controls. Histological differences were observed, however, in both the anterior pituitary glands and the adrenal glands of the bulls.

4. Although moderate prepubertal vitamin A deficiency did not completely inhibit sperm production, fertility and reproductive capacity appeared to be seriously impaired, since sexual drive and spermatogenesis as measured by laboratory procedures and histological examination were reduced. The possibility that prepubertal damage may be permanent, especially when cystic pituitaries develop or total blindness occurs, should not be overlooked.

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THE EFFECT OF MILKING AT SHORT INTERVALS WITH AND WITHOUT INJECTIONS OF OXYTOCIN[1]

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Kellner (7) long ago demonstrated an increased production of milk per unit of time by decreasing the milking interval. Many methods since have been used to ascertain the course of milk secretion. Tgetgel (10) measured the increase in pressure within the mammary gland during the intervals between milkings. His data show that the pressure increases slowly after milking, but rises quite rapidly toward the approach of the next milking. Isaachsen (6) attempted to measure the course of milk secretion by canulating the mammary gland over a 24-hour period. The container into which the catheter drained was emptied at hourly intervals. The flow of milk from hour to hour was quite variable.

Filipovic (2) milked cows at one-half and hour intervals during the periods between normal milkings. The yields that were obtained were quite irregular. He interpreted the data as showing a peak secretion occurring at about 4 hours after the last normal milking.

Ragsdale *et al.* (8) and Garrison and Turner (4) studied the rate of milk secretion by milking cows at increasing intervals of from 1 to 36 and 1 to 24 hours, respectively. These authors concluded that the rate of milk secretion is quite constant at milking intervals of 12 hours.

Gasnier *et al.* (5) milked cows at intervals ranging from 0.5 to 16 hours. Their conclusions were that milk production was proportional to the length of the interval during the first 6 hours, and for longer intervals milk production varied logarithmically.

Shaw (9), milking cows at 2-hour intervals with the aid of oxytocin, found that milk secretion was uniform from milking to milking. According to the theory proposed by Ely and Petersen (1), it is necessary for the cow to respond to the stimulus of milking by releasing the hormone, oxytocin, thereby causing the "let-down" phenomenon before the preformed milk in the cow's udder can be removed.

EXPERIMENTAL METHODS

Eight Holstein cows were selected from the University herd, on the basis of level of production and stage of lactation, to form four pairs such that the two individuals of a pair were as nearly alike as possible.

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Records of the milk weights and fat tests on each cow were kept for a 6-day pre-experimental period. During the subsequent 6 days, or experimental period, the cows were milked 12 times in 24 hours, beginning at 6 a.m. Thus the 6 a.m. milking constituted the beginning of a new day. On the injection day for a given group, each cow of the group was injected with 10 I.U. of oxytocin immediately prior to each milking.

The design of the experimental period is illustrated as follows:

Day of experimental period	1	2	3	4	5	6
Group I	Pre-injection	Injection	Inter-injection	Injection	Post-injection	Post-injection
Group II	Pre-injection	Pre-injection	Injection	Inter-injection	Injection	Post-injection

Days 2, 3, 4, and 5 of the experimental period constituted a "switch-back" design with respect to injection. However, the results obtained from cows of Group I on the sixth day of the experimental period have no counterparts among the results obtained from the cows of Group II. Also, the results from the cows of Group II have no counterparts obtained from Group I on the shift from twice-a-day to 12-times-a-day milking on the first day of the experimental period.

Records were kept of the pounds of milk produced and the per cent of fat of the milk for each cow at each of the 12 daily milkings during the 6 days of the experimental period.

The cows were returned to their regular twice-a-day milking schedule following the 6-day experimental period. Records again were kept of the pounds of milk and per cent of fat for each cow at each milking for 7 days subsequent to the experimental period. The day immediately after the experimental period was considered a transitional period, and the next 6 consecutive days were termed the post-experimental period.

One of the necessary conditions for valid use of analysis-of-variance methods is uniform variability of all the measurements involved in the analysis. The tests of significance associated with analysis-of-variance procedures are meaningless when this condition is not fulfilled, at least approximately. Since data for the respective cows differ in variability, and the variability is greatly reduced during the period of injection and greatly increases after cessation of injection, the data were analyzed by the rank method (3), as no common transformation would "bring the data into line".

RESULTS

Comparison of the Pre-experimental, Experimental and Post-experimental Periods

Average daily production of milk. Table 1 gives the 6-day averages of the milk production of each cow for each of the three 6-day periods involved

TABLE 1

Average daily production of milk by each cow during each of the three periods of the study

Group	Cow	Period					
		Pre-experimental		Experimental		Post-experimental	
		lb.	Rank	lb.	Rank	lb.	Rank
I	228	36.3	2	42.6	3	34.0	1
	282	38.6	2	43.8	3	37.6	1
	290	28.7	2	32.9	3	27.6	1
	190	11.9	2	16.8	3	6.6	1
II	242	41.0	2	49.2	3	37.7	1
	258	41.6	2	48.2	3	36.0	1
	307	26.1	2	30.2	3	24.5	1
	174	13.6	2	17.1	3	12.8	1
Av. standard error		0.44		1.16		0.52	

in the study. The averages for each cow were ranked 1, 2, or 3 in increasing order of magnitude. The most conspicuous feature of the results is the complete consistency of the ranks, as the average daily production of every cow was greatest during the experimental period and least during the post-experimental period. Since the probability of eight sets of three ranks each being completely consistent by chance alone is 1/279,936, it is clear that the particular pattern of complete consistency observed is significant. The average standard error shown in table 1 for the experimental period is more than double the values shown for each of the other two periods. This increase in variability, which is significant at the 0.01 probability level, largely is due to the vicissitudes of milk production on the days following injection days, which greatly over-balanced the greater uniformity of milk production on injection days.

TABLE 2

Weighted average per cent fat of milk produced by each cow during each of the three periods of the study

Group	Cow	Period					
		Pre-experimental		Experimental		Post-experimental	
		%	Rank	%	Rank	%	Rank
I	228	3.51	2	3.37	1	3.85	3
	282	3.77	2	3.54	1	4.26	3
	290	3.64	2	3.48	1	4.05	3
	190	4.66	2	3.75	1	5.04	3
II	242	3.57	2	3.31	1	4.10	3
	258	3.48	2	3.25	1	4.10	3
	307	3.89	2	3.63	1	4.78	3
	174	3.34	1	3.40	2	3.59	3
Av. standard error		0.069		0.121		0.173	

Average per cent fat. The average per cent of fat for each period is presented in table 2, together with the relative ranks. These are weighted averages of the figures on per cent of fat for the respective milkings and are not simple arithmetic means.

For each cow the highest average per cent fat occurred during the post-experimental period, in which the average daily production of milk was least. Also, with the exception of cow no. 174, the lowest average per cent of fat occurred during the experimental period, when the average daily production of milk was greatest. Since the probability of obtaining by chance alone either a perfect negative correlation between two groups of eight sets of three ranks each or a case only one permutation distance from a perfect negative correlation is 1/559,872, the observed negative correlation is statistically significant. Average standard errors shown at the bottom of table 2 increase

TABLE 3

Average daily production of fat by each cow during each of the three periods of the study

Group	Cow	Period					
		Pre-experimental		Experimental		Post-experimental	
		lb.	Rank	lb.	Rank	lb.	Rank
I	228	1.27	1	1.43	3	1.31	2
	282	1.46	1	1.55	2	1.60	3
	290	1.04	1	1.14	3	1.12	2
	190	0.55	2	0.63	3	0.33	1
II	242	1.46	1	1.63	3	1.55	2
	258	1.45	1	1.56	3	1.48	2
	307	1.02	1	1.09	2	1.17	3
	174	0.455	1	0.58	3	0.458	2
Av. standard error		0.1067		0.0762		0.0486	

uniformly from the pre-experimental period through the post-experimental period. This trend is significant at the 0.01 probability level. From these data it is not possible to determine whether this is due to injections and their after-effects, or to the effect of 12 milkings per day during the experimental period and the after-effects of this procedure, or both.

Average daily production of fat. Table 3 gives the 6-day averages of the fat production (in pounds) of each cow for each of the periods of the study, as well as the relative ranks of the three averages for each cow. The figures for average daily production of fat do not form as clear-cut patterns as the figures shown in tables 1 and 2. However, for each cow the average daily production of fat was higher during the experimental period than during the pre-experimental period, and, with the exception of cow no. 190, it also was higher during the post-experimental period than during the pre-experimental period. Since the probability of obtaining by chance alone a pattern

equally or more consistent is 2/2,187, the pattern observed may be regarded as significant. The average standard errors of total fat production shown at the bottom of table 3 decrease uniformly from the pre-experimental period through the post-experimental period, a trend that is significant at the 0.01 level and in the opposite direction from that observed in the average standard errors of per cent fat.

Effect of Injections

Level of total milk production. The total production of milk (in pounds) of each cow on each of the 12 daily milkings during the 6-day experimental

TABLE 4

Milk production (exclusive of the two "transitional" milkings) of each cow for each day of the experimental period

Day	Type	Group I, cow no.:							
		228		282		290		190	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	30.0	1	35.3	3	25.4	1	11.3	1
2	Injection	35.1	4	37.5	4	26.8	2	12.7	2
3	Inter-injection	34.0	3	41.6	5	26.9	3	18.1	5
4	Injection	38.5	5	42.6	6	30.8	6	18.3	6
5	Post-injection	40.0	6	30.9	1	27.7	4	14.4	3
6	Post-injection	33.5	2	34.5	2	28.4	5	15.0	4
Day	Type	Group II, cow no.:							
		242		258		307		174	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	40.0	3	37.1	1	26.7	5	14.2	3
2	Pre-injection	43.5	5	39.6	3	26.4	4	14.9	5
3	Injection	42.5	4	40.1	4	25.6	3	14.5	4
4	Inter-injection	37.7	1	39.1	2	24.3	2	12.8	2
5	Injection	46.3	6	46.5	6	27.3	6	16.8	6
6	Post-injection	39.1	2	42.2	5	22.6	1	12.7	1

period is shown in figure 1. Without exception, the milk production on the first milking of an injection day either equaled or exceeded the maximum production of the other milkings on the same day, and without exception the milk production on the first milking of a control day following an injection day was below average for the day, although not the minimum for the day in all cases. Consequently, the first two milkings of each day were excluded from the analyses to eliminate the "transitional milkings" from one treatment to the other.

Table 4 gives the total production of milk of each cow for the last ten milkings of each day of the experimental period, and the relative rank of each day's production. The rankings for both groups exhibit significant divergence from chance arrangements, the divergence being significant at the

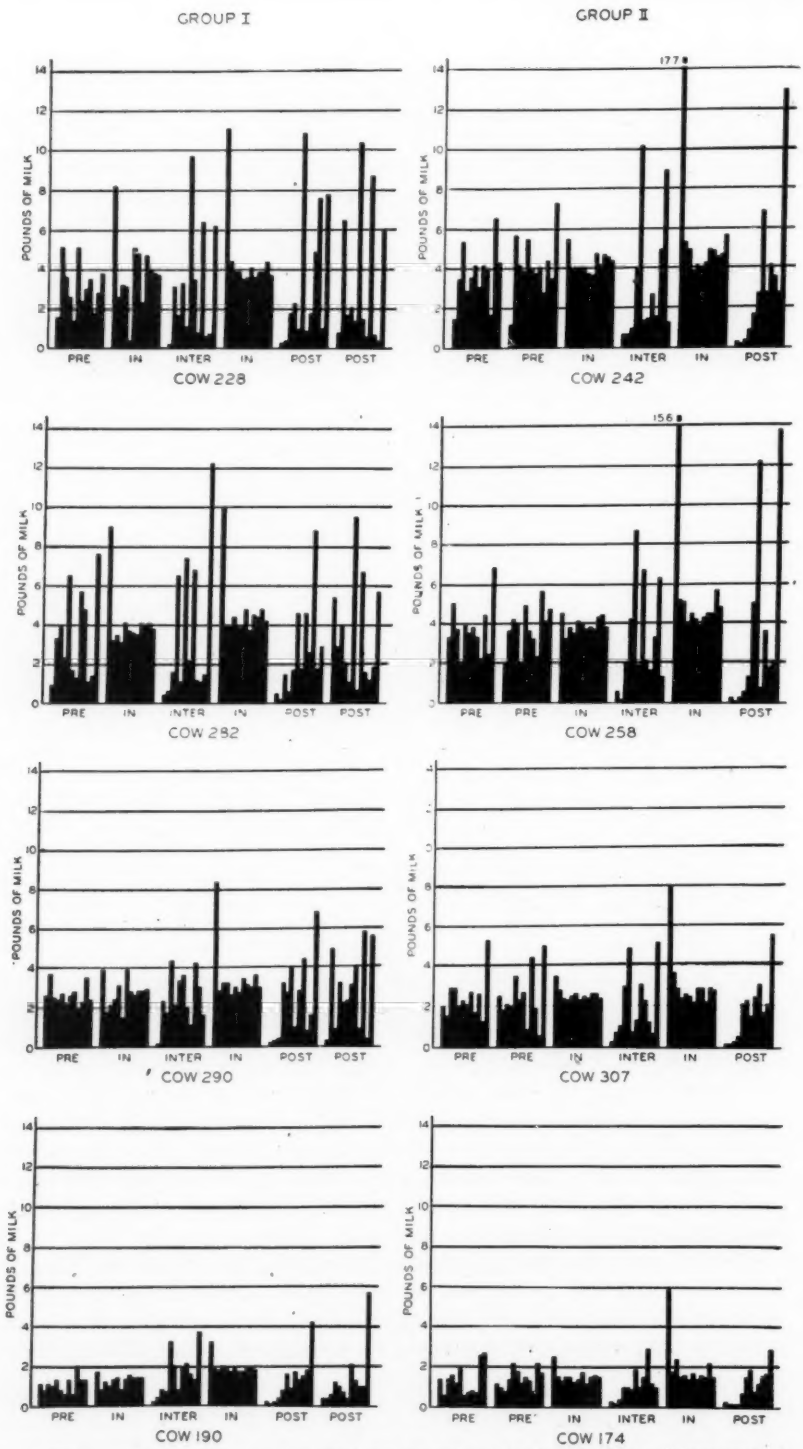

FIG. 1. Total milk (in lb.) of each cow on each of the twelve daily milkings on each of the 6 days of the experimental period.

0.05 level in the case of Group I and at the 0.01 level in the case of Group II. The only features common to the two parts of the table, however, appear to be the above-average production of the second injection day, and the fact that production on the second injection day exceeded production on the first injection day without exception.

Variability of total milk production. Examination of the various parts of figure 1 shows that, neglecting the initial milking on injection and post-injection days, milk production in general was more uniform from milking

TABLE 5

Measures of the variability of the milk production from milking to milking (exclusive of the two "transitional" milkings) of each cow for each day of the experimental period*

Day	Type	Group I, cow no.: 228		282		290		190	
		lb.²	Rank	lb.²	Rank	lb.²	Rank	lb.²	Rank
1	Pre-injection	10.2	2	55.6	4	1.5	2	1.6	3
2	Injection	17.4	3	1.0	1	3.6	3	0.4	2
3	Inter-injection	86.6	4	132.5	6	11.7	4	9.2	4
4	Injection	0.6	1	1.3	2	0.8	1	0.1	1
5	Post-injection	116.0	5	51.4	3	34.6	6	11.2	5
6	Post-injection	120.1	6	78.3	5	32.1	5	21.5	6
Day	Type	Group II, cow no.: 242		258		307		174	
		lb.²	Rank	lb.²	Rank	lb.²	Rank	lb.²	Rank
1	Pre-injection	16.4	4	17.1	4	10.9	3	5.4	5
2	Pre-injection	13.9	3	11.4	3	17.8	4	2.2	3
3	Injection	1.2	1	0.7	1	0.1	1	0.2	1
4	Inter-injection	100.7	5	59.9	5	25.6	6	4.3	4
5	Injection	2.7	2	2.6	2	0.8	2	1.1	2
6	Post-injection	121.7	6	217.6	6	20.1	5	6.2	6

* Sums of the squares of the deviations of the pounds of milk of the 10 non-transitional milkings from the corresponding averages were used to indicate the variability in the pounds of milk.

to milking on injection days than on pre-injection days, and generally more variable on post-injection days than on pre-injection or inter-injection days.

Table 5 gives the value of the sum of squared deviations of the milk yields of each cow for the ten non-transitional milkings from the average yield of these milkings on the day concerned. The relative ranks of the six figures for each cow also are given. The measures of milking-to-milking variation of milk production clearly are consistent with the visual impression obtained from examination of figure 1; they are extremely small for injection days and extremely large for post-injection days. Without further statistical analysis, it is clear that injection results in a significant reduction in milking-

to-milking variation of milk production and a significant increase in variability on days following injection.

Level of per cent of fat. The per cent fat of the milk produced by each

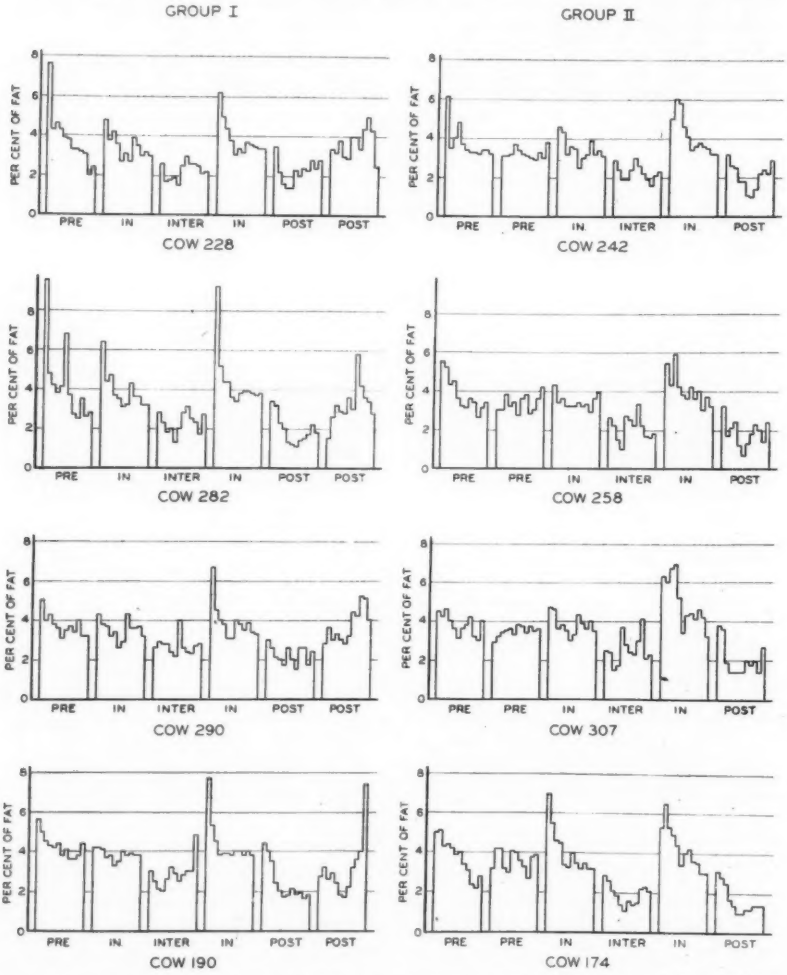

FIG. 2. The per cent fat of the milk of each cow on each of the twelve daily milkings on each of the 6 days of the experimental period.

cow on each of the 12 daily milkings during the 6-day experimental period is shown in figure 2. The per cent of fat of the milk produced on the first two (or three) milkings of an injection period generally was above average,

with the per cent fat on the first injection milking generally the highest, except for cows of Group II on their second injection day. The per cent fat of milk produced on a day immediately following an injection day tends to be less than the per cent fat produced on either an injection day or on a pre-injection day.

Table 6 gives the per cent fat of the milk produced by each cow on the ten non-transitional milkings (*i.e.*, excluding the first two "transitional" milkings) of each day of the experimental period. The relative ranks of the six figures given for each cow also are shown. The pattern of ranks for the

TABLE 6

Per cent of fat of milk produced (exclusive of the two "transitional" milkings) by each cow on each day of the experimental period

Day	Type	Group I, cow no.:							
		228		282		290		190	
		%	Rank	%	Rank	%	Rank	%	Rank
1	Pre-injection	3.40	4	3.41	3	3.57	4	4.06	5
2	Injection	3.28	3	3.59	4	3.43	3	3.78	3
3	Inter-injection	2.38	1	2.41	2	2.74	2	3.16	2
4	Injection	3.55	5	3.87	6	3.60	5	3.95	4
5	Post-injection	2.39	2	1.58	1	2.20	1	1.86	1
6	Post-injection	3.73	6	3.79	5	3.95	6	4.53	6
Day	Type	Group II, cow no.:							
		242		258		307		174	
		%	Rank	%	Rank	%	Rank	%	Rank
1	Pre-injection	3.55	5	3.43	5	3.79	5	3.36	3
2	Pre-injection	3.32	4	3.41	4	3.54	3	3.59	4
3	Injection	3.27	3	3.35	3	3.65	4	3.63	5
4	Inter-injection	2.15	2	2.06	2	2.66	2	1.80	2
5	Injection	3.87	6	3.93	6	4.51	6	3.97	6
6	Post-injection	2.08	1	1.75	1	1.91	1	1.28	1

cows of Group I and Group II differs from random arrangements, to extents that are significant at the 0.01 probability level. A common feature of the rank patterns for these two groups of cows is the extreme below-average rank of the per cent fat produced on a day immediately following an injection day. A contrasting feature, common to both groups, is the above-average rank of per cent fat produced on the second injection day. Examination of graphs of per cent fat per milking for each cow on each day of the experimental period (fig. 2) indicates the presence of considerable variation in per cent fat from milking to milking within a day.

Sums of the squares of the deviations of the per cent fat of the ten non-transitional milkings from the corresponding averages were used to indicate the variability in per cent of fat among the pre-injection, injection, and post-

TABLE 7

Measures of the variability of the per cent fat of the milk produced on each milking (exclusive of the two "transitional" milkings) by each cow on each day of the experimental period*

Day	Type	Group I, cow no.: 228		282		290		190	
		%²	Rank	%²	Rank	%²	Rank	%²	Rank
1	Pre-injection	5.77	6	14.52	6	1.32	2	1.06	3
2	Injection	2.25	3	2.45	3	2.04	4	0.48	2
3	Inter-injection	1.84	2	2.82	4	2.32	5	5.43	5
4	Injection	1.20	1	0.90	1	1.13	1	0.40	1
5	Post-injection	2.46	4	1.58	2	1.54	3	2.76	4
6	Post-injection	5.38	5	7.48	5	7.10	6	24.74	6
Day	Type	Group II, cow no.: 242		258		307		174	
		%²	Rank	%²	Rank	%²	Rank	%²	Rank
1	Pre-injection	2.22	4	2.90	3	2.37	4	5.90	6
2	Pre-injection	0.80	1	2.09	2	0.22	1	2.32	3
3	Injection	1.28	2	0.72	1	1.22	2	2.58	4
4	Inter-injection	1.49	3	4.02	5	6.15	5	1.52	1
5	Injection	5.72	6	5.72	6	8.70	6	5.42	5
6	Post-injection	3.50	5	2.96	4	1.66	3	1.70	2

* Sums of the squares of the deviations of the per cent fat of the 10 non-transitional milkings from the corresponding averages were used to indicate the variability in per cent of fat.

TABLE 8

Total fat produced by each cow each day (exclusive of the two "transitional" milkings) during the experimental period

Day	Type	Group I, cow no.: 228		282		290		190	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	1.02	3	1.20	3	0.91	3	0.46	2
2	Injection	1.15	4	1.35	5	0.92	4	0.48	3
3	Inter-injection	0.81	1	1.00	2	0.74	2	0.57	4
4	Injection	1.37	6	1.65	6	1.11	5	0.72	6
5	Post-injection	0.95	2	0.49	1	0.61	1	0.27	1
6	Post-injection	1.25	5	1.31	4	1.12	6	0.68	5
Day	Type	Group II, cow no.: 242		258		307		174	
		lb.	Rank	lb.	Rank	lb.	Rank	lb.	Rank
1	Pre-injection	1.42	4	1.27	3	1.01	5	0.48	3
2	Pre-injection	1.44	5	1.35	5	0.94	4	0.535	5
3	Injection	1.39	3	1.34	4	0.93	3	0.527	4
4	Inter-injection	0.810	1	0.81	2	0.65	2	0.23	2
5	Injection	1.79	6	1.83	6	1.23	6	0.67	6
6	Post-injection	0.813	2	0.74	1	0.43	1	0.16	1

injection days. These measures of variation are presented in table 7 with the relative ranks of the six measures for each cow. The pattern of ranks for Group I and Group II differs from random arrangements to extents that are significant at the 0.01 and 0.05 probability levels, respectively.

Table 8 gives the total fat produced by each cow in the last ten milkings of each day of the experimental period, together with the relative ranks of the six figures given for each cow. The pattern of ranks of Groups I and II are very similar to the corresponding patterns for per cent fat given in table 6. Cessation of injections was followed by a slump in fat production, followed by a high on the second day after injection.

TABLE 9

Measures of the variability of the total fat produced on each milking (exclusive of the two "transitional" milkings) by each cow on each day of the experimental period*

Day	Type	Group I, cow no.: 228		282		290		190	
		lb.²	Rank	lb.²	Rank	lb.²	Rank	lb.²	Rank
1	Pre-injection	0.0198	3	0.0569	4	0.0022	1	0.0022	3
2	Injection	0.0165	2	0.0028	1	0.0050	3	0.0007	2
3	Inter-injection	0.0564	4	0.0980	5	0.0125	4	0.0204	5
4	Injection	0.0027	1	0.0036	2	0.0023	2	0.0002	1
5	Post-injection	0.0822	5	0.0138	3	0.0230	5	0.0034	4
6	Post-injection	0.2013	6	0.1549	6	0.0783	6	0.1408	6
Day	Type	Group II, cow no.: 242		258		307		174	
		lb.²	Rank	lb.²	Rank	lb.²	Rank	lb.²	Rank
1	Pre-injection	0.0212	3	0.0195	3	0.0215	4	0.00486	5
2	Pre-injection	0.0266	4	0.0156	2	0.0224	5	0.0033	4
3	Injection	0.0041	1	0.0017	1	0.0010	1	0.0007	1
4	Inter-injection	0.0513	5	0.0402	5	0.0257	6	0.0023	3
5	Injection	0.0161	2	0.0208	4	0.0095	2	0.00490	6
6	Post-injection	0.1043	6	0.0941	6	0.0160	3	0.0011	2

* Sums of the squares of the deviations of the pounds of fat of the 10 non-transitional milkings from the corresponding averages were used to indicate the variability in pounds of fat.

Table 9 gives for total fat the values of the measure of within-day variability (excluding the two transitional milkings) adopted in connection with per cent fat and total milk. In the case of total fat, however, the tendency for the variability to be below average on injection days and high on post-injection days is somewhat better defined than in the case of per cent fat, where no common feature presents itself.

DISCUSSION

Milking cows normally at 2-hour intervals resulted in extreme milking-to-milking variability in production. These results indicate that the cows

were incapable of responding at 2-hour intervals to the normal stimulus of milking by a "let-down" of milk. The milking-to-milking variability of production was greatly reduced when oxytocin was used to facilitate a "let-down".

Conclusions drawn by Ragsdale *et al.* (8), Garrison and Turner (4), and Gasnier *et al.* (5) may be in error if a "let-down" cannot be elicited at 0.5-1-, and 2-hour intervals, as multiples of production obtained at those intervals would not give a true trend of milk secretion over longer periods. The first milking on an injection day, with one exception, exceeded the maximum production of any of the other milkings of the same day, which indicates that all the milk being produced is not removed when cows are milked at short intervals without the use of oxytocin.

Turner (11) postulates that the pressure due to the accumulation of milk within the lumina of the alveoli becomes sufficiently great to prevent the normal excretion of milk by rupture of the cell membrane. At high pressures, only those constituents of milk which are permeable to the cell membranes pass out of the cells into the lumen of the alveolus. The fat globules which are in suspension are retained in the cells. According to Turner, this theory explains the decrease in the per cent of fat concomitant to a lengthened milking interval, and the increase in per cent of fat in advanced lactation and milk strippings. If this theory is correct, the milk produced at short milking intervals at a low alveolar pressure should contain a higher per cent of fat than milk produced at longer milking intervals. In our experiments the per cent of fat, with one exception, was least during the 12-time-a-day milking period, when according to Turner's theory the per cent of fat in the milk should have been highest. However, the daily fat production was greatest with two exceptions during the 12-time-a-day period as a result of an increased flow of milk and not an increase in per cent of fat.

The data indicate that milk secretion proceeds at a uniform rate when cows are milked at 2-hour intervals, which verifies the results obtained by Shaw (9). However, at variance with Shaw's data was an increase in total milk and fat production by a more frequent removal of milk.

SUMMARY

A complete removal of accumulated milk was not obtained at each 2-hour milking interval when cows were milked normally, resulting in variable production from milking to milking.

Injections of 10 I.U. of oxytocin just prior to each 2-hour milking interval greatly decreased the milking-to-milking variability of production.

When cows were milked 12 times a day, total fat production, with two exceptions, was greater than in the pre- and post-experimental periods when the cows were milked twice daily. The yield of non-fat milk constituents showed an even greater increase, resulting in a lowered percentage of fat, with one exception, during the experimental period.

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THE AMOUNT OF DDT FOUND IN THE MILK OF COWS FOLLOWING SPRAYING[1]

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When it was shown that DDT may be absorbed through the skin of animals (2, 5) and that animals fed massive doses of this material produced milk containing toxic doses of DDT (1, 6, 7), considerable interest was created in the possibility that cows sprayed with this insecticide for fly control might produce milk containing toxic amounts of DDT. To test this hypothesis a cooperative experiment was planned to show the effects of very heavy spraying and also the effects of the spraying schedule suggested for hornfly control in this area.

Sixteen cows were selected from the Experiment Station Dairy Herd in such a way that no two experimental cows stood side by side. They were divided into four lots, each containing two Holsteins and two Jerseys. Except for the spraying, experimental cows were handled in the same manner as the regular milking herd. No precautions were taken to prevent the animals from obtaining DDT by licking themselves or other test animals. The test animals were not segregated from the rest of the herd and were pastured, except during milking hours. Milk used as the control was secured from cows stanchioned in another section of the dairy barn. The udders of all cows were thoroughly washed before milking with a solution containing 200 p.p.m. available chlorine. The entire herd had been sprayed with 0.25 per cent DDT 2.5 months preceding the initial experimental spraying.

Lot I was sprayed daily with 2 quarts of 5 per cent water suspensible DDT spray per animal and lot II with 2 quarts of 1 per cent emulsion. (This latter lot was sprayed with 5 per cent emulsion for the first 4 days but it became necessary to decrease the concentration to avoid further "burning" by the solvent.) Lots III and IV were sprayed on the same day every other week with 2 quarts of 0.25 per cent DDT spray. Material for lot III was made of water suspensible powder and that for lot IV of an emulsion. All spraying was done with a 3-gallon compressed-air sprayer after the evening milking. An attempt was made to cover the entire animal each time. Spraying was started July 15 and discontinued August 30.

Milk samples were taken in the evening directly from the milking machine and were transferred to the Department of Agricultural Chemistry Research, where they were analyzed for DDT content. The samples of milk

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were stored in a refrigerator and the DDT separated from the milk and concentrated by a slight modification of the method of Schechter *et al.* (3). The color was developed by the procedure set forth by Schechter *et al.* (4). Color densities were determined in the earlier samples by using the Bausch and Lomb colorimeter. The later determinations were made with the Coleman spectrophotometer at 596 mμ. In every case milk free from DDT and milk containing known amounts of DDT were run as controls. Samples were taken at weekly intervals.

To obtain a more comprehensive picture of the elimination of DDT through the milk, a single animal, no. 17, was sprayed and sampled daily. The spray used was composed of 2 quarts of 5 per cent water suspensible DDT. This animal was sprayed first on August 10 and sprayed last on August 30.

TABLE 1

Amounts of DDT appearing in the milk of cows sprayed with DDT

No. of cow	Breed of cow	Type of DDT	Conc. DDT (%)	Interval between spraying[a]	Max. amt. DDT in milk in p.p.m.	Duration of DDT elimination in milk[b]
1	Jersey	Wettable powd.	5.0	Daily	20	126[f]
2	Holstein	Wettable powd.	5.0	Daily	18	21
3	Jersey	Wettable powd.	5.0	Daily	+	.....
4	Holstein	Wettable powd.	5.0	Daily	+	.....
5	Jersey	Emulsion	1.0[d]	Daily	19	56
6	Holstein	Emulsion	1.0[d]	Daily	19	56
7	Jersey	Emulsion	1.0[d]	Daily	+	.....
8	Holstein	Emulsion	1.0[d]	Daily	+	.....
9	Jersey	Wettable powd.	0.25	14 days	1.5	21
10	Holstein	Wettable powd.	0.25	14 days	Trace	7
11	Jersey	Wettable powd.	0.25	14 days	+	.....
12	Holstein	Wettable powd.	0.25	14 days	+	.....
13	Jersey	Emulsion	0.25	14 days	2.5	21
14	Holstein	Emulsion	0.25	14 days	0.20	7
15	Jersey	Emulsion	0.25	14 days	+	.....
16	Holstein	Emulsion	0.25	14 days	+	.....
17	Jersey	Wettable powd.[c]	5.0	Daily[e]	33.6	119[f]

[a] Sprayed from July 15 to August 30.

[b] The time in days elapsing between the initial positive analysis and the first negative one.

[c] Milk from this animal was analyzed daily for DDT.

[d] During the first 4 days these animals were sprayed with 5% DDT but it then was necessary to drop the concentration to 1% to avoid further injury.

[e] Sprayed from August 10 to August 30.

[f] End of lactation period.

RESULTS

Milk samples collected from experimental cows 3 and 10 days after spraying were entirely negative for DDT. All samples taken the seventeenth day after spraying was started were positive to qualitative tests, but no quantitative tests were run.[2] The following week quantitative tests indicated that

[2] To reduce the volume of chemical work, milk was analyzed from only one Holstein and one Jersey of each lot of cows after it was shown that the milk of all experimental animals contained DDT.

animals sprayed with 1 per cent emulsion were giving off 18 to 19 p.p.m. of DDT in the milk, while those sprayed with 5 per cent wettable powder produced milk containing 5–12 p.p.m. Animals sprayed with 0.25 per cent showed less than 1 p.p.m. The maximum concentration of DDT, 33.6 p.p.m., appeared in the milk 20 days after spraying started. At the end of their lactation period, 119 and 126 days after the final spraying, two cows still were giving off appreciable quantities of DDT. Samples of milk collected

TABLE 2

Parts per million of DDT appearing in milk of cow sprayed daily with 2 quarts of 5 per cent DDT

Date	Aug.	Sept.	Oct.	Nov.	Dec.
1	.....	24.2	4.0	3.4	Trace
2	.....	16.9	3.7	3.9	2.1
3	.....	11.9	2.9	.....	2.7
4	.....	14.9	2.8	4.6	2.8
5	.....	12.6	2.6	4.0	2.1
6	.....	11.2	3.8	4.2	2.9
7	.....	8.7	3.1	3.8	2.9
8	.....	9.4	2.4	3.1	2.8
9	.....	7.5	3.3	3.4	2.8
10	.....	7.0	1.8	4.8	2.8
11	.....	6.0	3.3	4.2	3.0
12	.....	8.1	2.1	5.0	2.6
13	.....	3.2	1.9	3.0	.....
14	.....	7.5	1.7	4.6	.....
15	.....	.....	2.3	3.6	.....
16	2.0	5.4	2.5	3.1	1.8
17	3.0	7.8	1.3	3.9	.....
18	2.9	4.6	1.8	2.0	.....
19	2.9	4.2	1.2	1.2	1.3
20	10.1	2.1	1.1	2.0	.....
21	8.5	2.9	1.1	3.0	.....
22	15.0	2.8	1.4	3.2	.....
23	6.3	2.2	1.7	2.7	.....
24	8.1	3.1	1.4	2.6	.....
25	11.6	2.9	2.0	3.8	.....
26	8.4	2.6	1.0	4.3	.....
27	8.0	3.4	2.5	4.1	.....
28	5.3	3.0	3.2	3.5	.....
29	18.5	4.4	2.7	2.7	.....
30	33.6[a]	3.5	2.2	1.8	.....
31	24.0	.....	0.9	.....	.....

[a] Spraying discontinued.

from one cow immediately after calving and 24, 48, 72, and 168 hours later were negative for DDT.[3] Under the conditions of the experiment, the milk produced by Jerseys generally contained more DDT than that of the Holsteins. The details of this experiment are shown in table 1.

Table 2 shows the results of the detailed experiment involving a single cow sprayed and sampled daily. Six days after the initial spraying, 2 p.p.m. of DDT were found in the milk. Two weeks later 33.6 p.p.m. were found.

[3] This animal calved 217 days after her last spraying.

While spraying was in progress, analyses varied widely, perhaps due to contamination. After spraying was stopped, results were more uniform. More DDT appeared in the milk during the third month after spraying was stopped than during the second month.

BIOLOGICAL TESTS

Milk from test cows showing the highest quantity of DDT was fed to 12 young mice. They were forced to consume as much as possible by mixing all food with the milk and supplying this milk as the only liquid to drink for a period of 4 months. No demonstrable differences were noted in the test mice or their progeny when compared with control mice fed in a similar manner using normal milk.

Third instar larvae of mosquitoes, *Aedes aegypti* (Linn.) and *Culex quinquifasciatus* Say, were placed in dilutions of milk from cow no. 17 when it was showing the largest amounts of DDT. Larvae in both check and test milk were unable to live unless the concentration of milk was reduced below 50 per cent. Below this point larvae developed normally in both test and check series, and no evidence of DDT toxicity was noted. In addition, known amounts of DDT in acetone were added to milk diluted to half strength with water. The mixture then was homogenized to decrease the smothering effect due to the cream. No toxicity to larvae was noted in mixtures containing below 20 p.p.m. while above 50 p.p.m., all larvae were killed. In water, less than 0.1 p.p.m. kills all mosquito larvae. Little difference was noted in the susceptibility of *Aedes aegypti* and *Culex quinquifasciatus*.

Milk from cow no. 17 was supplied to cages of flies and mortality determinations were made after 72 hours. The death rates in the check cages were slightly higher than in the control lot, but no typical symptoms of DDT poisoning were found. Flies fed milk to which 50 p.p.m. DDT had been added lived slightly longer than flies from the same lot fed untreated milk.

DISCUSSION

Since it was the purpose of these experiments to demonstrate the amount of DDT that might appear in the milk of cows as the result of spraying, no precautions were taken to prevent the cows from licking themselves or other test animals. The data accumulated suggest that very small amounts of DDT may appear in the milk even though minimum spray schedules are followed. If very heavy applications are used, as much as 33.6 p.p.m. may be found in the milk at times. However, biological tests with mice, mosquito larvae, and flies suggest that even maximum amounts do not cause appreciable toxicity. When large quantities are used, DDT apparently is stored in the cow's body and eliminated in the milk for extended periods.

Biological tests have failed to demonstrate toxicity in milk from cattle

sprayed with DDT, even though they were treated with many times the amount that normally would be used for arthropod control. Excessive amounts of emulsion caused irritation, but no injury was noted in cattle sprayed with the same concentration of wettable powder DDT spray.

SUMMARY

Four lots of cattle were sprayed with DDT to determine the amount of this material that may appear in the milk following recommended and excessive treatment.

All animals tested excreted some DDT in the milk.

Animals sprayed with excessive amounts continued to eliminate DDT until the end of their lactation period, 119 and 126 days after the final spraying, but none after freshening.

Biological tests for toxicity of the milk to mice, mosquito larvae, and flies were negative.

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THE EFFICIENCY OF THE FARM-SIZE MECHANICAL SEPARATOR IN WITHHOLDING EXTRANEOUS MATTER FROM CREAM[1,2]

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The problem of extraneous matter in farm-separated cream is becoming increasingly important. In order to develop effective control measures, information is needed on the amount of contamination occurring at the various stages of production and marketing of cream. The possible sources of contamination of milk are fairly well established. The first stage of study on sources of sediment contained in cream when it arrives at the creamery would seem to be as the cream comes from the separator. The centrifugal action of the mechanical cream separator bowl together with the accumulation of separator slime during the separation process raised the question regarding the efficacy of the separator as a means of removing extraneous matter from cream during the separation process. The question also arises as to whether the mechanical cream separator will remove some types of material and not others. The amount of sediment found in cream as it comes from the separator will indicate whether primary emphasis should be placed on production practices prior to separation or on later stages of handling, if cream relatively free from sediment is to be obtained.

Boxell (1) has reported that cream obtained by mechanical separation on the farm had a lower sediment content on arrival at the creamery than did cream obtained by other methods. Experimental trials by the Research Committee of the American Butter Institute (5), in which split lots of the same milk were separated by mechanical separation, water dilution, and hand skimming, showed that the cream obtained by the different methods gave sediment tests scoring 1, 2, and 2+, respectively, by the American Butter Institute cream sediment standard (1946). In spite of the long-time, extensive use of mechanical cream separators on farms and the relatively long existence of the sediment problem in cream, no other published experimental data pertinent to the problem have been found.

Differences of opinion on the subject are indicated by the fact that a representative of one of the large manufacturers of cream separators (4) stated that the mechanical cream separator should not be expected to be an effective means of removing extraneous matter from cream. On the other hand, Judkins and Mack (3), in listing the advantages of the farm-size

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mechanical separator, state in their textbook that the cream is free from dirt because the separator acts as a clarifier. The objectives of the experiments herein reported were to determine the effectiveness of mechanical cream separation in preventing sediment in the milk from entering the cream under varying conditions and with different kinds of extraneous matter.

METHODS

In the laboratory trials two makes of farm-size, power-driven separators were used; one had a capacity of 800 lb. per hour and the other of 750 lb. per hour. The separators were washed, assembled, and flushed with warm water immediately prior to each use except in trials otherwise specified. Approximately 40 lb. of whole milk were used for most trials, since this amount was considered about average for Kansas cream-producing farms. Occasionally, larger quantities were separated as indicated. The milk was warmed to about 100° F., sampled for sediment testing, and separated. The milk sample was not taken in the customary off-the-bottom manner used on receiving platforms, but was obtained by stirring thoroughly and taking a pint sample. Where considerable farm contamination was present, no additional material was added to the milk; but when the milk was relatively clean, various amounts of dust, soil, manure, and combinations thereof were added prior to separation. One quart of sediment-free water was used to flush the separator bowl after separation was completed, by pouring the water into the supply tank. In some of the trials the flush water from the cream spout was collected separately, not being allowed to enter the cream. Slightly over 4 pints of cream and about one-half pint of cream flush were obtained from each separation. After separation, the separator parts were examined to determine where the extraneous material was lodged. With a few exceptions, sediment tests were made on 2 ounces, 1 pint, and 3 pints of the mixed cream, and on the cream flush when the latter was collected separately. The 2-ounce quantity was used since it is the one commonly tested in cream stations and is the amount upon which the American Butter Institute cream sediment standards (1946) are based. The 1-pint quantity was used because it is the amount often employed in plant tests and regulatory examinations. The 3-pint quantity represented most of the remaining cream from 40 lb. of milk and permitted a more critical evaluation of the amount of sediment in the cream. Standard 1¼″ lintine sediment test discs were used with a laboratory suction filtering device. Since the cream was sweet, only warm water was required to assist filtering. Sediment pads were examined both with and without the wide field microscope and scored according to the following modification of the American Butter Institute cream sediment standards, using plus or minus signs to indicate variations.

American Butter Institute cream sediment score:	1	2	3
Corresponding scores used in this study	1, 2, 3	4, 5, 6	7, 8, 9

To determine the situation under field conditions, samples were obtained at five cream-producing farms. In these farm trials the separator was prepared and operated by the producer. The milk was sampled from the separator supply tank into which it usually was strained. Two-ounce and 1-pint samples of the mixed cream were obtained for sediment testing. The flushing was done by the producer in his usual manner. Both milk and cream samples were sediment tested on the farm with one of the common types of cream sediment testers, warmed filtered water from the laboratory being used on the cream. Generally, the water used for flushing also was tested for sediment. Information was obtained relative to the age and general condition of the separator and the amount of milk sepa-

TABLE 1

Efficiency of the farm-size mechanical separator in withholding extraneous matter from cream

(Laboratory trials with the cream flush collected separately)

Trial	Lb. milk used	Material added to milk	Sediment score				
			Whole milk	Cream			Cream flush
			1 pint	2 oz.	1 pint	3 pints	½ pint
1	40	None	8	.....	.....	5	4
2	40	None	5	1	1	3	3
3	250	None	9	1	1	.....	.....
4	350	None	9	.....	3	.....	3
5	600	None	4	1	1	2	2
6	40	0.1 g. road dust	7	.....	4	5	2
7	40	0.1 g. road dust	7	1	1	3	4
8	40	0.15 g. dry manure	14*	.....	5	11*	8
9	40	0.05 g. dry manure	9	.....	.....	5	6
10	40	0.2 g. fresh manure	8	1	2	5	3
11†	40	0.2 g. fresh manure	8	1	2	5	5
12‡	40	0.2 g. fresh manure	8	2	5	5	5
13	40	0.1 g. soil	9	1	2	3	5
14†	40	0.1 g. soil	11*	3	3	5	7
15‡	40	0.1 g. soil	12*	3	5	7	7
Approx. av. score			9−	1+	3−	5	5−
Approx. A.B.I. score§			3+	1−	1	2	2

* Score approximated.
† Separator unwashed from previous trial (1 day).
‡ Separator unwashed from two previous trials (2 days).
§ American Butter Institute cream sediment standard (1946).

rated. The sediment pads were examined and scored by the method previously mentioned.

RESULTS

Results of laboratory trials in using mechanical separation as a means of withholding extraneous matter from cream when various amounts and types of material had been added to the milk are shown in table 1. In the trials involved, the flush water from the cream spout was collected and tested separately. Milk with high sediment scores purposely was used to

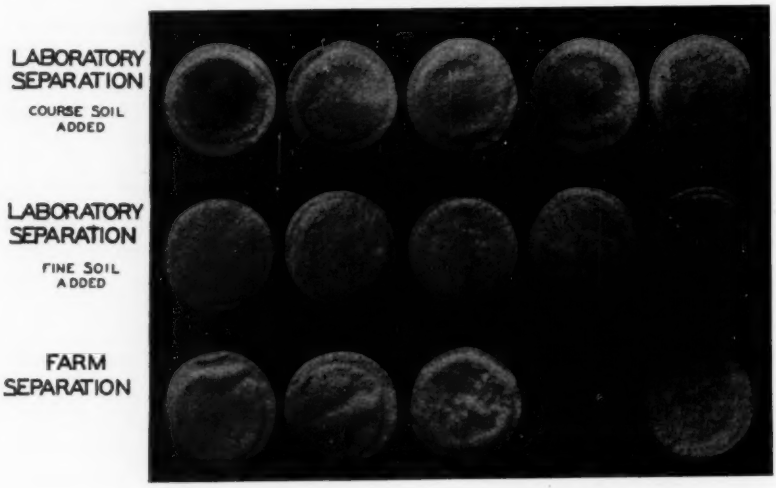

FIG. 1. Sediment tests of milk, cream, and cream flush from representative separation trials.

evaluate under extreme conditions the efficiency of the mechanical separation process in withholding extraneous matter. Thirteen lots had a sediment score equivalent to 3 or more by the American Butter Institute standard and would have been reject milk on the basis of sediment. The approximate average score was 9 – by the modified score used in this study and 3 + by the A.B.I. standard.

When sediment tests were made on the cream, the amount obtained from a 2-ounce sample was very small and usually was evident only on close macroscopic inspection, the average score being 1 + by the system used in this study and 1 – by the A.B.I. standard. As would be expected, the larger samples had a higher sediment score, the 1-pint samples averaging 3 – (A.B.I. score 1) and the 3-pint samples averaging 5 (A.B.I. score 2).

Considering the high sediment content of the milk used in these laboratory trials, with the flush water withheld from the cream, the results would seem to justify the conclusion that the mechanical separator was relatively effective in withholding from cream most of the sediment present in the milk. It resulted in cream of acceptable sediment score by prevailing standards. Representative results are shown in figure 1.

The 15 lots of milk involved in these laboratory trials included five lots to which no additional extraneous matter was added, two lots to which road dust was added, two lots to which dry manure was added, three lots to which fresh manure was added, and three lots to which light soil was added. Although comparison among trials using the same type of material was possible, it was difficult to make comparisons between trials with different types of sediment because of the difference in amounts used and the variation in size and number of particles involved. In general, however, when the added material contained a high proportion of large-sized, light weight particles (chaff, dried manure, and leaf fragments), more sediment escaped into the cream, while the heavier particles (sand, clay, and coal dust) were withheld.

As separator slime accumulates in the bowl, the separator conceivably might become less effective in withholding extraneous matter from the cream. To investigate this factor, three larger lots of milk (250, 350, and 600 lb. in trials 3, 4, and 5, respectively) with considerable farm contamination were separated and cream samples were obtained toward the end of the separation process. The sediment scores on the cream samples thus obtained were not significantly different from those in other trials where samples were taken from thoroughly stirred, entire lots of cream obtained from the separation of 40-lb. quantities of milk. The large quantities of high sediment-content milk used, which would represent the extreme in volume that might be expected under farm conditions, were not sufficient to cause enough accumulation of sludge in the bowl to impair the separator efficiency in removing extraneous matter. While cow hairs usually were present in the farm-contaminated milk, they rarely were found in the cream or cream flush samples tested for sediment. Hairs were found in the bowl sludge chamber; in instances where the hair contamination in the milk had been heavy, some were found near the cream ports in the lower discs.

To determine whether failure to wash the separator affects its efficiency in removing extraneous material, trials were conducted in which the same separator was used both clean and unwashed. Equal amounts of milk with specific amounts of added contaminating material were used (trials 10 to 15). After using the clean separator it was left unwashed one day before the next trial, and then left unwashed an additional day before using it again. Under the conditions of these trials the increases in the sediment

scores of the cream samples were not as marked as might be expected. It was noted that a slight amount of rust was expelled into the cream after the separator had remained unwashed and assembled. Rusted spots were evident in the machine when it was dismantled.

As previously mentioned, in the above trials the flush water expelled through the cream spout was kept separate from the cream. The sediment score of this cream flush was related to the sediment score of the original milk and to a lesser extent to the sediment score of the cream. The average score of the flush was 5 – (A.B.I. score 2) which was about the same score as on 3 pints of cream, although the cream flush involved only about one-half pint. Thus, on the basis of comparative volume, the cream flush contained about six times as much sediment as the cream.

TABLE 2

Efficiency of the farm-size mechanical separator in withholding extraneous matter from cream

(Laboratory trials using 40 lb. milk with the cream flush collected in the cream)

Trial*	Sediment score			
	Whole milk	Cream		
	1 pint	2 oz.	1 pint	3 pints
1	7	1	2	4
2	6	1	1	4
3	6	1	2	5
4	7	1	2	3
5	6	1	2	3
Approx. av.	6+	1	2	4–
Approx. A.B.I. score	2+	1–	1	2–

* In trials 1, 2, and 3, 0.15 g. of soil-manure mixture was added to each lot of milk. In trials 4 and 5 the milk contained only the original contamination.

In five trials (table 2) in which the cream flush was allowed to enter the cream, the average sediment score of 2-ounce samples was 1 (A.B.I. score 1–), of 1-pint samples it was 2 (A.B.I. score 1), and of 3 pints it was 4– (A.B.I. score 2–). These results compare favorably with those reported in table 1, in which the cream flush was kept separate from the cream, but the sediment score of the milk used was uniformly better. Although the cream flush apparently was not of sufficient volume to change materially the sediment score of the cream, the excessively high sediment content of the cream flush as shown in table 1 would indicate that withholding it from the cream would be of definite advantage in keeping sediment out of cream.

Since observations in previous trials had indicated that the separator removed most of the cow hairs present in the milk, seven laboratory trials were conducted to determine the efficiency of the separator in withholding

rodent hairs added to the milk, because of the possibility of this type of contamination on the farm (2) (table 3). The degree of contamination of the milk and the cream was measured by counting the number of rodent hairs found on the sediment pads. The results showed that the separator definitely was effective in withholding rodent hairs from the cream, but an occasional hair was found in the larger samples, indicating that some may escape into the cream. Assuming the number of hairs found in the pint samples of milk to be representative, there would be an average of about 360 hairs in the 40-lb. lots of milk separated. Since practically all the cream from each lot of milk was used in the three different sediment tests

TABLE 3

Efficiency of the farm-size mechanical separator in withholding rodent hairs from cream
(Laboratory trials with rodent hairs added to 40 lb. of milk before separating)

Trial	No. of rodent hairs on sediment pads				
	Whole milk	Cream			Cream flush
	1 pint	2 oz.	1 pint	3 pints	½ pint
Cream flush collected separately					
1	22	....	....	1	0
2	6	0	0	0	1
3	8	0	0	2	1
Av.	12	0	0	1	< 1
Cream flush collected in cream					
4	6	0	1	1	....
5	11	0	0	1	....
6	4	0	0	1	....
7	5	0	0	1	....
Av.	6	0	< 1	1	....

on the cream, it may be concluded that the separator removed better than 95 per cent of the hairs. In the three trials where the cream flush was kept separate from the cream, the number of hairs in the flush was not markedly different from that in the cream. Also, the number of hairs found in cream which included the cream flush was little different from that in the cream where the flush was held separately. Examination of the interior of the separator bowl showed the hairs to be entrapped in the sludge chamber. These results would indicate that rodent hairs found in farm-separated cream would consist primarily of contamination after separation.

The preceding studies of the efficiency of the cream separator in removing various types of extraneous matter were conducted under more ideal conditions than generally would be found on the farm. Therefore, eight farm trials were made as a check against the laboratory findings. The separators were of smaller capacity than those used in the laboratory, and ranged from 4 to 8 years of age. In each instance they had been washed since

previous use. The milk separated generally was cleaner than that selected for use in the laboratory.

In the farm trials the separator again had a considerable clarifying effect on the cream (table 4). In one trial, rodent-type hairs were found in the milk but none was found in the cream samples tested. However, the removal of sediment in the farm trials was not so complete as in the laboratory trials. Considering the better quality of milk involved, this suggests the need for

TABLE 4

Efficiency of the farm-size mechanical separator in withholding extraneous matter from cream

(Farm trials)

Trial	Approx. lb. milk	Sediment score				
		Whole milk	Cream		Cream flush	Flush water
		1 pint	2 oz.	1 pint	½ pint	1 pint
Cream flush collected separately						
1	40	8	4	4	5	.....
2	15	2	1	1	3	5
3	40	5	1	2	4	6
4	70	6	2	2	3	.....
Approx. av.	40	5+	2	2+	4−	5+
Approx. A.B.I. score		2	1	1	2	2
Cream flush collected in cream						
5	45	6	2	5	.....	7
6	40	6	3	4	.....	3
7	30	1	2	3	.....	6
8	40	4	2	4	.....	4
Approx. av.	40	4+	2+	4	.....	5
Approx. A.B.I. score		2−	1	2	.....	2

careful attention to separating conditions to obtain best results. The cream flush contained considerably more sediment than the cream portions, considering the volume involved, but generally less than the cream flush in the laboratory trials. The influence of collecting the cream flush in the cream was most marked in the pint samples, resulting in about twice as much sediment as when the flush was withheld. The sediment score on the water used for flushing was higher than on the cream and about the same as on the original milk. This may have had an influence on the increased sediment content of the pint samples when the cream flush was included. Nevertheless, under farm conditions the cream from the separator was reasonably

clean as measured by the sediment test on the commonly used 2-ounce size cream samples.

CONCLUSIONS

1. Under laboratory conditions and using high sediment content milk, cream from farm-size mechanical separators was practically clean as judged by present widely used methods of testing, having a no. 1 sediment score or better (A.B.I. standard). Even under farm conditions where no special precautions were taken, reasonably clean cream was obtained with no. 1 sediment scores on 2-ounce samples.

2. In general, when the extraneous matter in the milk contained a high proportion of large-sized, light weight particles, more sediment escaped into the cream, while the heavier particles were withheld. Cow hairs rarely were found in the cream.

3. The separation of large quantities of milk, which would represent the extreme in volume that might be expected under farm conditions, did not impair the separator efficiency in removing extraneous matter, even though the milk used had a high sediment content.

4. In the laboratory, failure to wash the separator did not result in as marked an increase in the sediment content of the cream as might be expected.

5. The farm-size mechanical separator was very effective in withholding rodent hairs from cream.

6. The cream flush contained proportionately more sediment than the cream, which would suggest that withholding it from the cream would be a possible measure in reducing sediment.

7. In the practical control of sediment in cream where mechanical separators are used, it would appear that, along with reasonable precautions in the separation process, more emphasis should be placed on attention during later stages of handling.

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FROZEN HOMOGENIZED MILK. III. STABILITY OF MILK SOLIDS DISTRIBUTION IN FROZEN HOMOGENIZED MILK

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Previous studies (1, 3, 4, 5, 7) have shown that freezing and storage temperature affect the physical character of homogenized milk. Later studies (2, 6) showed that when homogenized milk is frozen, the solid components tend to concentrate in the lower portions of the sample. Changes in the temperature at which frozen homogenized milk was stored did not, however, materially affect the chemical composition of the different sections of quart samples.

The results reported by the authors (2) were based in part on samples of homogenized milk frozen and stored under conditions simulating those encountered in transferring milk from land storage to storage on hospital ships. The results obtained on the chemical analysis of some of the individual samples indicated that the chemical constituents may continue to shift after the milk is frozen. Since the literature does not contain information regarding the movement of the chemical constituents in frozen homogenized milk, the present study was undertaken to determine whether or not the solid constituents do continue to shift after the milk is frozen.

PROCEDURE

Four groups of homogenized milk samples with a fat content of 3.8 per cent and packaged in quart paper containers by a commercial dairy in Washington, D. C., were frozen at −11.5° C., −17.8° C., −27.5° C., and −36° C. and held at these respective temperatures until they were removed for chemical examinations. The temperature of storage remained constant for all groups except one. Within 3 days after the milk was placed in storage at −17.8° C., the temperature rose to −16.7° C., and for the next 10 days it varied from −16.7 to −15.0° C. Furthermore, during the entire storage period there were times when the temperature rose as high as −12.2° C. To insure that initial freezing had taken place, a sample of each group was removed at the end of the third day. Subsequent samples were removed at 10-day intervals. All samples were divided, while frozen, into two equal halves and allowed to thaw at room temperature. Both the top and bottom sections were analyzed for protein, fat, total solids, lactose, and chlorides by

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TABLE 1

Chemical analyses of different sections of homogenized milk frozen and held at constant temperatures

Storage time (days)	% protein (N × 6.38)		% fat		% total solids		% lactose		% chlorides	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
A. Frozen and held at – 11.5° C.										
3*	2.74	3.91	2.85	3.94	10.06	14.03	3.94	5.38	0.084	0.116
13	2.54	4.17	2.64	4.15	9.21	14.94	3.56	5.71	0.077	0.123
25	2.98	4.22	2.80	4.24	9.28	15.10	3.54	5.86	0.076	0.124
34	2.69	3.97	2.87	4.04	9.95	14.38	4.01	5.67	0.083	0.118
43	2.66	3.92	2.78	3.85	9.68	14.04	3.84	5.44	0.090	0.115
54	2.45	4.22	2.59	4.22	8.78	15.04	3.33	5.78	0.073	0.124
64	2.50	4.25	2.61	4.19	8.95	14.98	3.53	5.82	0.072	0.121
74	2.49	4.25	2.56	4.18	8.78	14.95	3.41	5.72	0.070	0.122
84	2.50	4.30	2.55	4.10	8.89	15.11	3.46	5.93	0.074	0.123
96	2.46	4.25	2.55	4.15	8.79	15.01	3.59	5.99	0.072	0.123
104	2.43	4.17	2.54	4.02	8.88	14.83	3.55	5.86	0.072	0.120
113	2.57	.....	2.62	3.95	9.36	14.66	3.64	5.63	0.075	0.118
B. Frozen and held at – 17.8° C.										
3*	2.75	3.96	3.00	4.21	10.01	14.41	3.83	5.61	0.077	0.114
13	2.81	3.89	3.04	4.15	10.29	14.30	3.92	5.51	0.081	0.112
23	2.75	3.99	2.98	4.20	10.02	14.68	3.78	5.65	0.078	0.115
34	2.73	3.95	2.98	4.22	10.09	14.61	3.77	5.61	0.079	0.114
43	2.68	4.00	2.91	4.35	9.80	14.74	3.75	5.66	0.078	0.116
52	2.79	3.94	3.04	4.17	10.18	14.36	3.83	5.52	0.081	0.114
63	2.83	3.93	2.99	4.15	10.29	14.43	3.90	5.51	0.081	0.112
73	2.78	3.96	3.03	4.17	10.19	14.42	3.94	5.61	0.081	0.113
83	2.66	4.07	2.88	4.23	9.73	14.65	3.70	5.79	0.077	0.115
93	2.69	4.04	2.92	4.20	9.82	14.81	3.83	5.75	0.075	0.115
103	2.78	3.63	2.87	3.72	9.93	15.07	3.79	5.88	0.077	0.118
C. Frozen and held at – 27.5° C.										
3*	2.88	3.98	2.96	4.01	10.23	14.47	3.96	5.73	0.082	0.112
13	2.76	4.04	2.88	4.08	9.89	14.70	3.67	5.84	0.077	0.116
25	2.57	3.88	3.14	3.96	10.93	13.99	4.26	5.37	0.097	0.112
34	2.98	3.74	3.13	3.88	10.91	13.69	4.23	5.40	0.092	0.112
43	2.85	3.94	2.94	4.00	10.08	14.52	3.85	5.84	0.079	0.126
54	2.97	3.89	3.04	4.03	10.74	13.87	4.14	5.31	0.090	0.113
64	3.05	3.86	3.15	3.87	10.64	13.90	4.07	5.45	0.082	0.119
74	3.01	3.87	3.06	3.88	10.68	13.70	4.08	5.35	0.089	0.112
84	3.05	4.04	3.09	3.91	10.87	13.74	4.27	5.32	0.091	0.112
96	2.99	3.88	3.08	3.91	10.63	13.88	4.02	5.30	0.089	0.113
104	3.04	3.85	3.08	3.86	10.87	13.65	4.35	5.37	0.092	0.112
113	2.96	3.86	3.08	3.92	10.38	14.03	3.79	5.47	0.081	0.120
123	2.85	4.00	2.93	4.00	10.01	14.41	3.88	5.90	0.081	0.121
133	3.01	3.86	3.07	3.92	10.53	13.71	3.96	5.40	0.087	0.111
144	2.98	3.83	3.11	3.89	10.51	13.89	4.05	5.60	0.084	0.120
154	2.74	3.98	2.89	4.06	9.83	14.48	3.78	5.97	0.078	0.124
D. Frozen and held at – 36° C.										
3*	2.97	3.94	3.21	4.20	10.84	14.35	4.07	5.46	0.086	0.108
13	2.88	3.92	3.16	4.19	10.80	14.34	4.06	5.50	0.085	0.112
24	2.91	3.88	3.19	4.17	10.73	14.37	4.11	5.56	0.085	0.112
33	2.87	3.89	3.11	4.16	10.54	14.32	4.07	5.49	0.085	0.112
42	2.94	3.86	3.16	4.01	10.83	14.21	4.14	5.40	0.086	0.110
53	2.93	3.88	3.13	4.12	10.75	14.24	4.08	5.42	0.086	0.111
63	2.78	3.97	3.02	4.24	10.35	14.68	4.09	5.68	0.085	0.116
73†	2.93	3.92	3.17	4.19	10.67	14.40	3.99	5.41	0.087	0.113

* Initial.

† Mechanical failure of deep freezer occurred shortly after the 73rd day.

methods described in a previous report (2). After the samples had thawed, they were preserved by adding 1 ml. of formalin.

EXPERIMENTAL RESULTS

When homogenized milk was frozen and held at constant temperatures, *i.e.*, −11.5, −27.5, and −36.0° C., and at approximately −17.8° C., the concentration of milk solids was greater in the bottom section of the quart container than it was in the top section. The quantity of milk solids found in the top section ranged from 2.43 to 3.05 per cent protein, 2.54 to 3.21 per cent fat, 8.78 to 10.93 per cent total solids, 3.33 to 4.35 per cent lactose, and 0.070 to 0.097 per cent chlorides. In the bottom section the milk solids ranged from 3.63 to 4.30 per cent protein, 3.72 to 4.35 per cent fat, 13.65 to 15.11 per cent total solids, 5.30 to 5.99 per cent lactose, and 0.108 to 0.126 per cent chlorides.

After the milk was initially frozen, the quantity of each of the solid constituents found in the top and bottom sections remained practically constant (table 1). When the milk was frozen and held at −11.5° C., the greatest differences between the initial analysis and any subsequent analysis of the top and bottom sections, respectively, were as follows: protein, 0.31 and 0.39; fat, 0.31 and 0.30; total solids, 1.28 and 1.08; lactose, 0.61 and 0.61; and chlorides, 0.014 and 0.008. When the milk was frozen and held at approximately −17.8° C., the greatest differences were: protein, 0.09 and 0.33; fat, 0.13 and 0.49; total solids, 0.28 and 0.66; lactose, 0.13 and 0.27; and chlorides 0.004 and 0.004 for the top and bottom sections respectively. When the milk was frozen and held at −27.5° C., these differences were: protein, 0.31 and 0.24; fat, 0.19 and 0.15; total solids, 0.70 and 0.82; lactose, 0.39 and 0.43; and chlorides, 0.015 and 0.014. With milk frozen and held at −36.0° C., the differences in the solid constituents were: protein, 0.19 and 0.08; fat, 0.19 and 0.19; total solids, 0.49 and 0.33; lactose, 0.08 and 0.22; and chlorides, 0.001 and 0.008.

These differences from the initial analyses did not progressively increase or decrease with an increase in the storage time. This shows that the milk solids did not continue to shift after the initial freezing of the milk and that these slight differences may be attributed to experimental error in dividing the frozen sample into top and bottom sections or in some instances to the fact that the milk had separated to such an extent that it was impossible to obtain a representative sample.

It further can be determined from table 1 that after the milk was frozen initially, the quantity of each of the solid constituents found in the top and bottom sections was in close agreement with the average analysis (based on analyses made after different storage periods). The difference between the average and the initial analyses ranged from 0 to 0.16 and from 0.02 to 0.24 for the protein, from 0.03 to 0.19 and from 0.04 to 0.15 for the fat, from

0.02 to 0.84 and from 0.01 to 0.73 for the total solids, from 0.01 to 0.32 and from 0.03 to 0.35 for the lactose, and from 0 to 0.007 and from 0 to 0.005 for the chlorides in the top and bottom sections, respectively. This close agreement also shows that the milk solids did not continue to shift after the initial freezing of the milk.

CONCLUSION

When homogenized milk was frozen, the concentration of the milk solids in the lower portion of the sample took place during the freezing process and apparently there was no further movement of these solids after the milk was frozen.

The authors wish to express their appreciation to Edith May Giltner and Elmina Faulkner for their assistance with the analytical determinations.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

274. Animal Nutrition. Second Edition. LEONARD A. MAYNARD. 494 pages. $5.00. McGraw-Hill Book Co., New York. 1947.

The second edition of this well-established and excellent text follows closely the pattern of the first, which was published in 1937. The major purpose of the book, according to the author, is to teach how the facts of nutrition are being learned experimentally and how they find application in practical livestock feeding.

Like the original text, the new edition contains 16 chapters outlining the principles of nutrition and their applications in feeding practice. These deal, respectively, with the expanding field of nutrition, the animal body and food, physiochemical bases of life process, metabolism of the carbohydrates, lipids, proteins and the inorganic elements, vitamins, feeding experiments and determination of digestibility, nutritional balances, measures of food energy, maintenance, growth, reproduction, lactation, and work. The book also contains an entirely new feature, namely, a list of visual aids.

Extensive revisions and additions have been made to bring the subject matter up to date through March, 1946. Because of wartime conditions, most of the literature citations refer to work done in the United States. Much new information is presented about the proteins, minerals, and vitamins. The discussions of cobalt, manganese, and others of the so-called trace mineral elements in animal nutrition have been greatly expanded. Newly discovered rôles of carbohydrates and fats in animal nutrition are discussed. New facts are presented concerning the nutrient, mineral, and vitamin requirements of animals and about the comparative value of various feedstuffs for supplying those needs. Modifications of many ideas previously held are presented as based on recent experimental evidence.

The book is very well written, well illustrated, and well arranged. The author has done an excellent job of eliminating extraneous material. In spite of the fact that nutrition is to a large extent a chemical process, the subject matter is presented so clearly as to be readily understood even by those with little or no training in this field. Throughout the text, the author presents facts on the problems confronting the field of nutrition, on special applications to human nutrition, and on pioneer contributions of many individuals. These all help to add interest and value to the book. Cross references and the references listed at the end of each chapter help to increase the usefulness of the text as a source of information. T.W.G.

BACTERIOLOGY

275. Studies on Coliform Organisms in Dairy Products. L. BUCHBINDER AND EDYTHE ALFF, Bureau of Laboratories, Dept. of Health, New York City. Jour. Milk and Food Technol., *10*, 3: 137–148. May–June, 1947.

A survey of the coliform incidence in pasteurized milk in New York City was made for the purpose of establishing coliform standards. The question of heat resistance of coliforms in milk is of little significance.

The following conclusions were reached: (a) the reports in the literature on heat resistance of coliforms frequently are fragmentary and inconclusive; (b) many of the reports are based on laboratory results and not in a milk plant itself; and (c) differences in techniques employed in the study, such as partially submerged tubes, an unspecified number of organisms used, and, in many instances, a reporting of greater numbers of heat resistant organisms than normally would be found in raw milk, give a wide variation in the results.

The authors attempted to carry out three approaches to the problem. First, they determined the frequency with which coliforms are present in milk just leaving the holder in a pasteurizing plant. In many instances as few as five coliforms per liter of milk were found. Repeated tests over a period of several months showed that coliforms rarely escape destruction in the holder, being detected in only one of some 400 liters of milk sampled. Secondly, in only about 2% of the samples studied in a survey of the raw milk supply of New York City over a period of 1 year did the coliform count exceed 100,000 per ml. Thirdly, the authors found that when raw milk was pasteurized by the partial submergence technique, 7.5% of 482 samples showed coliforms. The presence of survivors was unrelated to the initial numbers present in the raw milk. The same method was used to test 18 pure cultures of the survivors in a total of 21 trials. The cultures each were tested in four decimal dilutions falling within the range of coliforms found in natural raw milk. Of the 84 tubes, 46 (55%) were positive. The survivors seemed to be distributed by chance among the several dilutions. Ten of the same strains were investigated in some 36 tests, using the complete-submergence, sealed-tube method, with the initial numbers ranging from 3 million to 396 million per ml., and thus much greater than would be expected in normal raw milk. No coliforms were recovered after 30 minutes. Shorter time intervals showed 8 of 25 cultures survived after 0 minute, 3 of 25 after 5 minutes, but none after 10 or 15 minutes. These results indicate that coliforms do not survive the time and temperature of laboratory pasteurization when the proper technique is used.

H.H.W.

276. What Is Meant by Coliform Bacteria. R. N. DOETSCH, Dept. of Bact., Univ. of Maryland, College Park. Milk Plant Monthly, *36*, 1: 89–91. 1947.

Coliform organisms are defined as "all aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacteria which ferment lactose with the production of gas". They are members of a large group of bacteria which have many characteristics in common. The presence of coliform bacteria in milk indicates improper pasteurization or post-pasteurization contamination. Dairy bacteriologists use all the coliform organisms as indicators of faulty procedure. Several methods are described for detecting coliform bacteria in dairy products. G.M.T.

277. Coliform Bacterial Contamination of Milk and Milk Products. Practical Problems and Their Solution. R. P. MYERS, Natl. Dairy Products Corp., Research Laboratories, Baltimore, Md. Milk Plant Monthly, *36*, 2: 30–33. 1947.

The coliform test, widely used in the dairy industry by plant operators, is particularly helpful in checking the efficiency of cleaning and sterilizing procedures. Experience in plant pasteurization of milk has demonstrated rather conclusively that coliform bacteria do not survive proper pasteurization, either by the holding or short-time method. Thus, if coliform bacteria are found, post-pasteurization contamination of the milk has resulted. The sources of coliform contamination may be as follows: (a) improperly sterilized equipment, (b) contamination of equipment when assembled, (c) condensate dripping from pipes, etc., (d) handling equipment which contacts dairy products, (e) air contamination, (f) contamination of pasteurized milk with improperly pasteurized milk, (g) vacuum lines at the bottle fillers, (h) contaminated colors, flavors, and fruits, particularly in ice cream, (i) rubber gaskets and packing glands, (j) dead-end tees, and (k) seepage through plug caps. Most effective sterilization is brought about by combination of both hot water and chemicals. Coliform bacteria grow at relatively low temperatures. Thus, the tests should be made on the fresh product rather than on a sample stored for some time in the refrigerator. The coliform tests when intelligently used in the plant aid in locating the points which need more attention in sanitation. G.M.T.

278. An Occurrence of Udder Infections Caused by a Thermoduric Streptococcus. J. S. BRYAN, Walker-Gordon Laboratory Co., Plainsboro, N. J. Milk Plant Monthly, *36*, 3: 32–33, 92. 1947.

Many thermoduric bacteria come from the udder of the cow. Cases are cited in which udders were infected by thermoduric bacteria on inadequately washed milking machines, resulting in high thermoduric counts in the milk.

Recommendations in the control of thermodurie streptococci in a herd or milk supply are: (a) constant vigilance and checking to discover infected cows and keeping milk of such cows out of the supply, (b) careful cleaning of milking machines to prevent spreading of organisms to other cows, and (c) careful cleaning and sterilizing of the pasteurizing and bottling equipment. G.M.T.

BUTTER

279. The Washing of Butter and Its Effect on Curd Content and Quality. E. G. PONT. Austral. Council Sci. & Indus. Res. Jour., *19*, 4: 432. 1946.

The presence of increased curd in the range 1.1% for washed butter and 1.52% for unwashed butter is not a determining factor in keeping quality. Unwashed butter involves adverse factors not determined in this investigation. Out of 74 churnings of butter, only two unwashed samples graded higher by one point after 1 week's storage. As the storage period advanced, the washed butter scored higher in most instances. After 3 months' storage, washed butter lost 1.23 points in grade while unwashed butter lost 1.71 points. There was no relationship for the inferior keeping quality of the unwashed butters and curd content, initial quality, and bacteria content. H.P.

280. Increased Costs of Operating Today's Butter Business. P. POTTER, Paul Potter & Associates, Chicago, Ill. Natl. Butter and Cheese Jour., *38*, 7: 46. July, 1947.

Responses to questionnaires representing 79 butter manufacturing plants show increased costs. These costs increased from 1940 to 1945 as follows: manufacturing costs, 51%; print room, 41%; overhead, 30%; and total costs, 41% (not including selling expense). Estimated increases in procurement cost averaged about 42%. The total unit cost of manufacturing, printing, and overhead increased about 1.4 cents. Increased costs per unit were due to reduced volume as well as to higher costs of labor and materials. W.V.P.

281. Dasher for Churns. H. R. MARSHALL. U. S. Patent 2,421,798, June 10, 1947 (3 claims). U. S. Patent Office Off. Gaz., *599*, 2: 233. 1947.

The surfaces of a blade for a rotary churn dasher are designed to improve the churning operation. R.W.

CHEESE

282. The Control of the Early Gas Defect in Cheese Caused by the Coli-Aerogenes Bacteria. TH. E. GALESLOOT, Netherlands Milk and Dairy Jour., *1*, 1: 33–42. 1947.

The early gas defect in cheese could be controlled with either KNO_3 or KNO_2. These compounds must be added in an amount sufficient to delay gas production from lactose until gas production can be checked sufficiently by the development of acid by the lactic acid organisms. $KClO_3$ also was suitable for controlling the early gas defect in cheese; a sufficient amount must be added so that the gas-forming organisms can reduce this compound until the lactic acid bacteria have increased sufficiently to inhibit the gassy fermentation. The objectionable flavor caused by the coli-aerogenes bacteria was not as pronounced when $KClO_3$ was used as when KNO_2 or KNO_3 was used. The author favors the use of $KClO_3$. F.J.B.

283. Cottage Cheese Manufacture. Part 1. Its Problems and Possibilities. W. I. TRETSVEN, Dairy Advisory Service, Chicago, Ill. Milk Plant Monthly, *36*, 5: 24–27, 32. 1947.

Production of cottage cheese is increasing rapidly since the quality has improved. This item is economical, is generally liked, and is profitable for the dairy manufacturer. Methods of manufacture are presented in considerable detail. Difficulties sometimes encountered are discussed and remedies suggested. The writer believes that the production of cottage cheese is plagued with superstitions and undesirable cure-alls, which may be wasteful, illegal, or questionable. With general improvement in quality, increased consumption may be expected. G.M.T.

284. Cottage Cheese Manufacture. Part 2. Creaming and Packaging of Cottage Cheese. W. I. TRETSVEN, Dairy Advisory Service, Chicago, Ill. Milk Plant Monthly, *36*, 6: 26–29, 32. 1947.

Since cottage cheese is mostly retailed as a packaged product, several problems of creaming and packaging cottage cheese are present. Creaming mixtures vary markedly in the percentage of fat. Details are given relative to the creaming of cottage cheese. Hand packaging procedures are criticized because of lack of sanitation, lack of uniformity, high labor costs, obsolescence, and lack of dependability of labor. Precautions must be taken to reduce contamination of cottage cheese. The yeast and mold content is a fair index of the sanitary condition of the product. A phosphatase and sediment test are essential to determine the effectiveness of pasteurization and general cleanliness. G.M.T.

285. Manufacture of Cheese. W. FALHNDRICH. U. S. Patent 2,423,300, July 1, 1947 (2 claims). U. S. Patent Office Off. Gaz., *600*, 1: 121. 1947.

A perforated tube is placed in a position approximating the axis of cheese at the time of manufacture, to facilitate drainage of the whey from the center, thereby improving the texture of the cured cheese. R.W.

CHEMISTRY

286. The Mechanism of the Oxidant Effects of Commercial Salt and Water in Butterfat. G. L. HILLS AND J. CONOCHIE. Austral. Council Sci. & Indus. Res. Jour., *19:* 414–429. 1946.

The catalysis of the oxidation of the fat in cold storage butter by salt and acid present in the water phase was studied at 40° C. using hydrogenated peanut oil to give solidity to the fat and to maintain the necessary fine moisture distribution. Dispersed water alone in butterfat accelerated oxidation. Catalysis of fat oxidation by commercial salt in acid solution was found to be due to the combined effects of halide and hydrogen ions. The effect is a direct one between acid salt-solution and fat; the presence of other butter constituents is not necessary. The mechanism suggested and supported by experimental evidence involves the formation by reaction between fat-hydroperoxide and hydrogen and chloride ions of chlorine, which then activates further oxidation. The previously reported oxidant effects of traces of magnesium chloride in commercial salt, when the salt is dispersed in the dry state in fats and oils, can be explained similarly. In butterfat, glyceryl monostreate added at the rate of 0.1 to 1.0% was found effective in stopping the reaction at the interface between the fat and salt solution. H.P.

287. Note on the Effect of Phosphatide on the Ferric Thiocyanate Method of Estimating Peroxide in Fats. G. L. HILLS AND R. WILKINSON. Austral. Council Sci. & Indus. Res. Jour., *19*, 4: 430. 1946.

Milk phosphatide at a concentration of 0.05% was found to interfere with the ferric thiocyanate test for peroxide groups in fats. This does not cause difficulty in the application of the test to dairy products other than dried buttermilk when benzene-methanol is used as the solvent. Acetone will not dissolve phosphatide to cause interference. H.P.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

288. Concentrated Milks. B. H. WEBB, Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Dept. of Agr. Milk Plant Monthly, *36*, 1: 44–46, 48, 50, 52. 1947.

Described briefly are the history of the concentrated milk industry, the

composition of various concentrated milks, their methods of manufacture, their nutritive values, and the economic factors necessary for the successful establishment of a condensery, namely, location in the dairy community, location in a small town, good transportation facilities, abundant water supply and good drainage, an available market for the products, and sufficiently large capital. G.M.T.

289. **From Pan to Package. The Importance of Cooling Process in the Manufacture of Sweetened Condensed Milk.** O. F. HUNZIKER. Milk Plant Monthly, *36*, 6: 74–76, 78–79. June, 1947; Natl. Butter and Cheese Jour., *38*, 7: 40. July, 1947.

The cooling process is one of the most important steps in the entire manufacture of sweetened condensed milk, for the type and number of sugar crystals will be determined largely by the treatment of the product during the cooling period. Sweetened condensed milk with very smooth texture and free from sugar crystal deposits is accomplished through mass crystallization. Seeding is indispensable in securing mass crystallization, the logical seed material being powdered lactose. The pulverized lactose may be added at the rate of 4 to 6 ounces of seed lactose per 1,000 lb. of original fluid milk. Several methods of cooling may be employed, each of which has advantages and disadvantages. G.M.T.

290. **The Future of Dry Milk.** S. O'DEA, Production and Marketing Administration, U. S. Dept. of Agr. Milk Plant Monthly, *36*, 1: 30–31, 64. 1947.

While the utilization of dry milk and nonfat dry milk solids has been high during the war years and many new uses for them have been found, the long-run post-war demand for milk powder is uncertain. Development of markets for nonfat dry milk solids is more imperative than for any other manufactured dairy product. G.M.T.

291. **Desiccating Method and Apparatus.** G. TOOBY. (Assigned to Richard C. Lilly.) U. S. Patent 2,422,706, June 24, 1947 (10 claims). U. S. Patent Office Off. Gaz., *599*, 4: 593. 1947.

A spray drier designed for milk is described. The milk is introduced into a stream of hot air traveling toward the periphery of a conical-shaped chamber. The denser dried milk particles are removed from the bottom of the cone-shaped chamber, while the moisture-laden air leaves through a duct at the top to enter a cyclone collector. R.W.

DISEASES

292. The Prevention, Control and Treatment of Mastitis. C. S. BRYAN, Dept. of Surgery and Medicine, Michigan State College, East Lansing. Milk Plant Monthly, *36*, 2: 97–98. 1947.

Mastitis is a very general term which refers to any inflammation of the udder regardless of the cause and severity of the condition. The most common cause of chronic infection is *Streptococcus agalactiae*. Available scientific data, substantiated by field trials, for the successful prevention and control of bovine mastitis involve good sanitary milking procedure, good sanitary herd management, early and accurate diagnosis of specific trouble, elimination by slaughter or complete segregation of cows with badly damaged udders, and early and proper treatment. G.M.T.

ICE CREAM

293. Technical Literature of Ice Cream for 1946. A. LEIGHTON, Bureau of Dairy Industry, U. S. Dept. of Agr. Ice Cream Rev., *30*, 11: 45. June, 1947.

This is the ninth annual review of ice cream literature by the author. In the current review, a total of 249 articles are cited, covering all phases of ice cream manufacture. The bibliography, classified according to subject matter, will afford a particularly valuable reference to ice cream manufacturers and technical or scientific workers. W.J.C.

294. Carboxymethylcellulose in Ice Cream. M. LANDERS. (Assigned to Lanco Products Corp.) U. S. Patent 2,423,600, July 8, 1947 (10 claims). U. S. Patent Office Off. Gaz., *600*, 2: 253. 1947.

Inclusion of a water-soluble salt of carboxymethylcellulose in an ice cream mix improves the texture of the frozen product without adversely affecting other characteristics. A mixture to be added to the mix consists of 30 parts sodium carboxymethylcellulose having a viscosity of 1,500 centipoises in 1% aqueous solution, 15 parts glycerol α-stearate γ-benzoate and 55 parts cane sugar. One part of this mixture is added to 200 parts of ice cream mix containing 10% fat. Less is required for mixes containing greater percentages of fat. All the claims include a mixed glycerol diester of a higher fatty acid and an aromatic carboxylic acid in addition to the water-soluble salt of carboxymethylcellulose. E.O.W.

295. Shrinkage in Ice Cream. C. D. DAHLE, D. J. HANKINSON, AND J. A. MEISER, JR. Dairy Dept., Pennsylvania State College. Milk Plant Monthly, *36*, 1: 32–33, 36–38. 1947.

Shrinkage in ice cream is the most important unsolved problem in the ice

cream industry today. Use of paper containers, continuous freezing, dry ice, high cabinet temperature, time of the year, and presence of free fatty acids, egg yolk, certain polyhydric alcohol esters, and certain sugars are among the contributing factors. A definite solution to the problem has not been found, and all the conditions thought to cause shrinkage may be present yet shrinkage may or may not occur. G.M.T.

296. Ice Cream Cabinet. R. J. SHARP. U. S. Patent 2,423,207, July 1, 1947 (1 claim). U. S. Patent Office Off. Gaz., *600*, 1: 98. 1947.

The sleeve of the cabinet is inclined toward the front of the cabinet to facilitate dipping of the ice cream. A pedal, pressed by the foot, causes the lid to slide upward, permitting access to the ice cream, the lid returning to the closed position on release of the pedal. R.W.

297. Customer Surveys as a Means of Checking up on Company Service. ANONYMOUS. Ice Cream Rev., *30*, 11: 46. June, 1947.

The results of a questionnaire submitted to customers at 13 Franklin Creamery Co. ice cream stores in Toledo and Cleveland, for the purpose of checking up on store operations, are summarized. A total of 2,095 replies were received, of which 76% contained comments of a favorable nature, whereas the remainder registered complaints on some phase of Franklin store operations. Specific things most frequently commented on favorably were: good quality ice cream, creamy texture of ice cream, good service, clean, attractive stores, good variety of flavors, availability of special molds and rolls, and convenient location of the store. Of the criticisms offered, poor service ranked first in number of replies, followed in order by unsatisfactory quality of ice cream, lack of table service, insufficient help, not open long enough, tables not cleaned often enough, no delivery service, not enough stores, insufficient variety of flavors, lack of parking facilities, and store not attractive. In the opinion of the manager, surveys of this nature provide some helpful information. However, the wide divergence of opinions expressed frequently was conflicting and did not permit clear-cut conclusions. W.J.C.

298. Dealer Price Education. V. M. RABUFFO, Ice Cream Trade Jour., *43*, 5: 40. May, 1947.

Suggestions are offered to dealers on how to establish retail prices. The price of fountain items, sodas, sundaes, and milk shakes should be about twice their cost. A satisfactory spread in the cost and selling price of ice cream sold in cones may be maintained by a slight reduction in the amount of ice cream served in cones in lieu of a price increase. A cone sold for double the price of a single cone should contain two scoops of ice cream and represent double value.

Dealers are advised to sell hand-dipped ice cream at 10 cents per pint over the price of a factory-filled package. A gross margin of 30 to 35% is suggested for factory-filled packages.

Retail prices should be set so that they will not retard sales. The price must be acceptable to the consumers, must bear the proper relation to prices of competing items, give the merchant a profitable return on his investment, and produce the greatest possible number of inventory turn-overs in any given period. The dealer should be shown that ice cream is a fast turn-over item in relation to most merchandise carried in stores. W.H.M.

MILK

299. Grade A Milk. H. A. RUEHE, Univ. of Illinois, Urbana. Milk Plant Monthly, *36*, 6: 30–32. 1947.

Considerable confusion exists in some localities as to what constitutes Grade A milk. The Milk Ordinance and Code formulated by the Public Health Service sets a standard of 50,000 per ml. for raw milk and 100,000 per ml. for raw cream of this grade. For Grade A pasteurized milk and cream, the bacterial plate count must not exceed 30,000 per ml. for milk and 60,000 per ml. for cream. Certain factors in the production of Grade A milk increase the costs of production so that it may not be economical for the small producer to strive to qualify for Grade A milk production.

G.M.T.

300. Procurement of Quality Milk. W. W. THOMPSON, Rochester Dairy Co-operative, Rochester, Minn. Milk Plant Monthly, *36*, 6: 103–104. 1947.

Procurement of quality milk depends in part on the efforts and relationships of a large number of people. Several basic principles are involved in carrying out a quality program. There must be an incentive for the producer to do a good job. The quality of the milk must be determined regularly. The producer must be kept informed of the job he is doing. Field service must be provided. These cannot be minimized. Cash premiums for producing quality milk have secured results. Likewise, the giving away of premiums for certain credits has been effective in producing quality milk.

G.M.T.

301. The Microscope in Controlling the Quality of Farm Milk. C. S. BRYAN, Dept. of Surgery and Medicine, Michigan State College, East Lansing. Milk Plant Monthly, *36*, 2: 52, 54, 72. 1947.

Microscopic examination may be used in detecting the source of contamination of poor quality milk. The procedure involves selecting a sample at the weighing vat in a sterile tube; preparing a standard smear; staining by

methylene blue, Mallmann's acid stain, Newmann's reagent, or Broadhurst's reagent; and examination of smears with the microscope, using oil immersion. Details of the various techniques are presented in considerable detail. G.M.T.

302. Control Practices Used in Supervision of Vitamin D Milk by City and State Milk Sanitarians. Report of Committee on Applied Laboratory Methods, K. G. WECKEL, Chairman, Univ. of Wisconsin, Madison. Jour. Milk and Food Technol., *10*, 3: 167–171. May–June, 1947.

Vitamin D fortified milk constitutes from 30 to 60% of the total fluid milk sales. It is important to the public that the desired potency be present in all vitamin D milk produced. Studies indicate that one out of every seven dairies produces milk below the desired potency. One out of every 20 samples collected by city sanitarians for bioassay purposes was found to be below standard.

Fortunately, inadequate fortification does not do the harm that could be done by inadequate pasteurization. Consumers as a rule pay a premium for vitamin D fortified milk and must rely upon assurance for labeled potency. In the light of this survey, many sanitarians should re-assay their procedure of supervision of vitamin D milk produced in their areas. H.H.W.

303. Developments and Sales Possibilities of Homogenized Milk. C. A. IVERSON, Dept. of Dairy Industry, Iowa State College, Ames. Milk Plant Monthly, *36*, 5: 64, 66, 68. 1947.

Although many developments have occurred within the last decade in the use and distribution of homogenized milk, probably its greatest development is yet to occur. Advantages claimed for homogenized milk are: (a) butterfat is uniformly distributed, (b) milk is uniform, thus appearing and tasting richer, (c) curd tension is reduced, (d) as a beverage it is a superior product, (e) for infant formulas, boiling, filtering and cooling can be eliminated, (f) certain defects are less pronounced in homogenized milk, (g) in the absence of cream there is no temptation for the operator to cut pasteurization temperature of holding time in order to protect creaming ability, and (h) homogenized milk does have sales appeal. On the other hand, homogenized milk does have its disadvantages, such as (a) no cream layer, (b) certain flavor defects, (c) sediment, (d) disadvantages in cookery, (e) cream line formation if process is improperly done, (f) utilization of returns, (g) increased costs, and (h) the homogenizer may be an additional source of contamination. The author believes that the main advantage which homogenized milk has is that people prefer it as a beverage to non-homogenized milk, and he concludes that homogenization is one of the forward-looking phases of the dairy industry. G.M.T.

304. What to Check and How to Check on Short-Time Pasteurizers. A. H. Rishoi, Cherry-Burrell Corp. Milk Plant Monthly, *36*, 5: 34–36, 38–41. 1947.

Attention is called to the many means and devices used to insure compliance with health department regulations and to some of the safeguards which have been built into the design and construction of short-time units. A short-time pasteurizer consists, essentially, of six parts: a float control tank, a regenerator section, a heater section, a holder tube, a flow diversion valve, and a cooler section. The general regulations which should be checked by the sanitarian with respect to milk flow are: (a) the pasteurized milk must be under greater pressure than the raw milk contained in the unit, (b) the milk pump must be sealed so the specified capacity is not exceeded, (c) a restriction is placed in the diversion flow line in order that the back pressure on the milk pump and milk in the holder pump will be the same during diversion, (d) the holding tube should slant upward continually from the point of entrance of the milk to the diversion valve, and (e) drainage to the float tank when the pump stops or when the raw milk line is disconnected from the regenerator outlet must be automatic.

Tests for measuring the time required for milk to pass through the holding tube or providing for injecting a test solution through a stopcock at the entrance and means for drawing liquid through a similar arrangement just ahead of the flow diversion valve have been devised. These test liquids involve a color solution or salt solution. The actual time for the solution to pass through the holding tube may be determined by observation or by electric current. Capacity of the unit is measured by the time required to fill a 10-gallon container. The function of the flow diversion valve is the very heart of the time-temperature control system. Tests on the flow diversion unit involve four steps, which include checking the recording thermometer and adjusting the controller by use of liquids at various temperatures. The paper is unusually informative and helpful to both operators and inspectors of short-time pasteurizers. G.M.T.

305. Experiences Using the Short-Time High-Temperature on Milk, Cream, Buttermilk, Cocoa and Cottage Cheese. M. M. Kloser, Bowman Dairy Co., Chicago, Ill. Milk Plant Monthly, *36*, 3: 38, 40–41, 86. 1947.

The high-temperature short-time pasteurizer has been used successfully in processing milk, cream, buttermilk, cocoa, and milk for cottage cheese. Temperatures employed for different products vary, depending upon the products being processed. Homogenized milk was pasteurized at 170° F. for 16.5 seconds, while the skim milk intended for buttermilk manufacture was heated to 185–190° F. Satisfactory results were obtained on the bases of bacteria, phosphatase, cream volume, and flavor. Greatest difficulty with

the use of the high-temperature short-time pasteurizer was with gaskets of synthetic rubber; these had to be replaced more often than the natural rubber gaskets, which had given 16 months of continuous service. G.M.T.

306. Milk Pail. R. J. GOODEN. U. S. Patent 2,422,541, June 17, 1947 (2 claims). U. S. Patent Office Off. Gaz., *599*, 3: 500. 1947.

A milk pail is described with a removable strainer and hinged cover. R.W.

SANITATION AND CLEANSING

307. Sanitary Standards for Dairy Equipment. E. H. PARFITT, Evaporated Milk Assoc., Chicago, Ill. Natl. Butter and Cheese Jour., *38*, 7: 48. July, 1947.

The Subcommittee on Sanitary Standards of the Dairy Industry Committee was appointed to collaborate with the International Association of Milk Sanitarians and the U. S. Public Health Service in developing standards of sanitary performance of processing equipment. Progress has been made, as evidenced by recommendations for fittings, published in the Jour. Milk Technol., Jan.–Feb., 1946, and for storage tanks, *ibid.*, May–June, 1946. Standards for transportation tanks, homogenizers, pumps, storage tanks, and farm milk utensils are being formulated. The success of these undertakings depends on the support of milk sanitarians and regulatory groups and on the education of buyers to specify 3A sanitary standards. W.V.P.

308. Sanitation and Plant Efficiency. C. E. WHITE, Kraft Foods Co., Chicago, Ill. Milk Plant Monthly, *36*, 2: 34–39. 1947.

Sanitation involves the struggle between man and the lower forms of life, such as yeast, bacteria, molds, insects and rodents, which may infest dairy plants. In checking a plant for sanitation, the operator should observe: plant and equipment cleanliness, plant operating methods, orderliness or plant housekeeping, and the condition of the building, premises, and plant equipment. The selection and training of plant personnel, and the proper selection of cleaners, sterilizers, and cleaning aids are of foremost importance. Fundamentally, cleaners serve three purposes—the dissolving of dairy soil, the reduction of interfacial tension between cleaning solution and soil, and the softening of the water through the formation of soluble compounds. Scheduled cleaning, *i.e.*, cleaning the equipment at the proper time, entails four fundamental preventive measures: (a) rinsing equipment between batches, (b) cooling the pasteurizer before and during emptying, (c) elimination of steam leaks, and (d) rinsing equipment immediately after emptying to remove all foam and dairy soil. Insect control is another factor in attaining plant sanitation; this involves eliminating breeding places, in-

sect proofing the building, and the correct use of insecticides. Rodent control also is necessary and includes elimination of breeding places, rodent proofing the building, and trapping. G.M.T.

309. Bottle Washing Problems. D. H. JACOBSEN, Cherry-Burrell Corp., Chicago, Ill. Milk Plant Monthly, *36*, 6: 42–44, 46–48. 1947.

The principal function of the bottle washer is to produce clean and sterile bottles. The problems in soaker bottle washing, according to their relative importance, are efficient sterilization, complete cleaning and bright appearance, prevention of etching of glass or color labeling, prevention of scale on the washer, and smooth mechanical operation or lubrication. Certain temperatures and concentration relationships must be maintained in bottle washing. Different states and municipalities have various standards for sterilization of bottles. Until uniform standards are established, milk plant operators should follow local standards on caustic concentration and then choose bottle washing compounds which provide the maximum in bottle cleaning efficiency. G.M.T.

310. Quaternary Ammonium Compounds in the Dairy Industry. M. E. DAVIS, Winthrop Chemical Co., Weston, Mass. Milk Plant Monthly, *36*, 3: 48, 50–52. 1947.

Quaternary ammonium compounds, of which "Roccal" was the first, have been introduced to the dairy industry with favorable results. The properties claimed for these compounds are: (a) rapid and complete killing action against bacteria even in low concentrations, (b) practically non-poisonous, (c) non-irritating to the skin, (d) odorless, (e) non-corrosive, (f) great stability, and (g) inhibit bacterial growth over long periods of time. In obtaining maximum efficiency with the use of these compounds, the following suggestions are helpful: clean all equipment; dip all small parts in disinfecting solution before assembly; leave loose all joints, pipe connections, dead-end tees, and valves in order that all surfaces may be contacted by the solution; and pump through the entire system a solution containing 200 p.p.m. Specific directions are given for the use of quaternary compounds in sanitizing milk cans, milking machines, and dairy utensils. G.M.T.

311. A Uniform System of Milk Inspection. W. D. TIEDEMAN, Bureau of Milk Sanitation, New York Dept. of Health, Albany, N. Y. Milk Plant Monthly, *36*, 3: 72, 74, 76. 1947.

A complete lack of uniformity exists in the system of milk inspection, not only by the municipalities but among various states. Attempts at securing legislation requiring a uniform inspection system in New York were not

successful in 1944 and again in 1945. If a reasonably efficient and uniform sanitary control over country sources of milk supply was maintained, duplication and much ill-feeling caused by stringent inspection in some areas and laxity of inspection in others would be eliminated. Indications are that in the future agreements between departments of health, agriculture, markets, and the dairy industry will be effected. G.M.T.

312. **Essentials of an Effective Farm Dairy Sanitation Program.** P. W. SCHERSCHEL, Laboratory and Field Service, Pure Milk Assoc., Chicago, Ill. Milk Plant Monthly, *36*, 6: 105–106. 1947.

An effective farm dairy sanitation program involves good cooperation between the receiving room, trucker, fieldman, and the producer. The fieldman has certain responsibilities in suggesting to producers ways and means of improving the quality of their milk. Producers as a rule want to market a good quality product, keep healthy cows, be clean, follow correct milking procedures, and control temperatures. Detailed directions for cleaning utensils, milking, and the care of the milk house and surroundings are given. G.M.T.

313. **Duties of a Fieldman.** W. J. BRAKEL, Dept. of Dairy Husb., Ohio State Univ., Columbus. Milk Plant Monthly, *36*, 4: 48, 50. 1947.

The duties of a fieldman are to satisfy his employer, to represent the firm by which he is employed, and to render service to the dairy producer. He often is the only representative of the organization purchasing the milk and as such should bring to the producer a favorable opinion concerning the plant. Retaining the goodwill of producers and increasing production are not difficult if the fieldman has proper attitudes and approaches a patron in a tactful, businesslike manner. Appealing to pride is an effective means of stimulating producers to milk quality; as a last resort, the producer may be advised that the market requires that certain changes be made in order to help in producing a higher quality product. The fieldman especially must be in close contact with receiving room operations, such as weighing, Babcock testing, and quality tests. Cans returned to the producers must be sanitary. The fieldman should spend sufficient time at the plant conducting quality tests and keeping records to enable him to have complete confidence in their accuracy. He should function as a service man, creating interest and enthusiasm for efficient production of high quality milk. He must be interested in his work, in its importance, and in keeping up-to-date with improved methods. G.M.T.

314. **The Use of DDT and Other Methods of Insect Control in Dairy Plants.** L. S. HENDERSON, Bureau of Entomology and Plant Quar-

antine, Dept. of Agr., Washington, D. C. Milk Plant Monthly, *36*, 3: 54–56, 60. 1947.

Preventive measures in controlling insects around food establishments may be even more important than the application of insecticides. Sanitation and rigid and thorough house-cleaning or plant-keeping are highly important. Sanitation should not end inside the plant but should include the premises as well. DDT is an extremely useful insecticide; however, there has been a great deal of hesitancy in recommending its use for fly control in rooms where food is processed or handled because the insects may, in the process of being destroyed, get into the equipment or food. DDT likewise is effective in roach control. A heavy roach population may appear a few days after its application because of the stimulation effected by DDT in bringing them out of their hiding places. If roaches still are present after 2 weeks, incomplete coverage usually is indicated. In using insecticides the importance of sanitation must not be minimized. G.M.T.

315. Insect and Rodent Control in Dairy Plants. F. A. KORFF, Bureau of Food Control, Baltimore City Health Dept. Milk Plant Monthly, *36*, 4: 98, 100. 1947.

The dairy industry has been fairly successful in the fight against microbial contamination of their products. Attention today is being given to the elimination of infestations from insects and rodents. Like bacteria, insects and rodents need food, water, and warm environment. By eliminating breeding places through sanitation and plant housekeeping, a great deal can be accomplished in keeping pests at a minimum. Recently emphasis has been placed on the importance of plant housekeeping, rather than on trapping and killing. The technical method of control consists of sealing up all openings where insects and rodents may enter and eliminating harboring places throughout the building. The biological method involves the reduction and elimination of all hiding and breeding areas. Effective results can be obtained by proper training of plant personnel and by the relocation of equipment so as to aid in cleaning and inspection operations. Sanitation is of utmost importance in the control of all dairy plant pests. G.M.T.

316. Insect and Rodent Control in the Plant. E. M. SEARLS, Sealtest, Inc., N. Y., N. Y. Milk Indus. Found. Assoc. Bul., *39*, 12: 350–354. March, 1947.

Insect and rodent control involves not only destruction of the pests but primarily preventing their entrance into food-handling establishments. The statement is made that about 80% of all convictions by the Federal Security Agency for adulteration of product published in the July, 1946, list were based on presence of insects or rodents or on that "might have been" clause.

At the present time there seems nothing more available or desirable than the insecticide DDT, which permits virtual proofing of a plant against insect occupation for long periods of time. Nothing approaching DDT for insects is available for rodents. It is questionable if poisoning rodents is advisable. Exclusion by rodent proofing is the first line of defense, with the old clap-trap style rat and mouse trap for those that get in. E.F.G.

317. New Chemicals for Insect Control on the Farm. E. M. SEARLS, Sealtest, Inc., N. Y., N. Y. Milk Indus. Found. Assoc. Bul., *39*, 9: 240–242. Feb., 1947.

Sanitation comes first in insect control. Have clean barns, clean premises, and clean, well-tended animals; then the application of insecticides will finish the job of insect control. Probably the most useful insecticide is wettable DDT applied with a knapsack-type sprayer according to the instructions of the manufacturer of the insecticide. Neither DDT nor any of the rest of these new insecticides can take the place of sanitation. E.F.G.

318. Ten Common Causes of Excessive Sediment. Part 1. Importance of Keeping Cows Clean. C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36*, 2: 64, 66. 1947.

Thousands of service calls on sediment trouble indicate that the ten most common causes of poor sediment tests at the receiving platform are: (a) cows, (b) milk cans, (c) improper use of filter disc and strainer, (d) failure to rinse strainer when filter disc is changed, (e) carelessness in storage of milk can lids, (f) milking machine suction cups allowed to get close to floor or bedding when being attached to the cow, (g) improper technique in pouring milk into strainer, (h) sediment in rinse water, (i) jarring lock strainer, and (j) single face discs placed in strainer with the gauze side up; the gauze should be down with the cotton side up. G.M.T.

319. Ten Common Causes of Excessive Sediment in Milk. Part 3. Proper Care of Milk Can Lids. C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36*, 4: 86, 88. 1947.

Careless handling and storing of milk can lids has been found to be one source of excessive sediment in milk. Fine dark specks found in milk were traced to pieces of bark falling from a tree into unprotected can lids which later were used without cleansing. G.M.T.

320. Ten Common Causes of Excessive Sediment in Milk. Part 4. Filter Disk Improperly Placed in Strainer. C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36*, 5: 82–83. 1947.

Fifty per cent of 2,700 milk producers who had milk returned because of undesirable sediment tests were not placing the filter disc into the strainer

properly; thus some of the milk by-passed the filter and was not strained. Every plant manager, fieldman, and milk hauler should learn how to place the disc into a strainer correctly and pass this information on to each of the milk producers. Both hands should be used in placing the disc. Unless the edges fit properly, the baffle plate or dome crumples the sediment disc, preventing a perfect seal. Dents in the bottom of strainer will cause similar by-passes. G.M.T.

321. Ten Common Causes of Excessive Sediment in Milk. Part 5. Failure to Rinse Strainer when Changing Disk. C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Plant Monthly, *36*, 6: 98–99. 1947.

Rinsing the strainer when changing the disc has been found to be a means of eliminating some of the sediment from milk. Unless this is done, some of the sediment taken out of the milk may lodge on the strainer under the new disc and be washed into the milk. G.M.T.

MISCELLANEOUS

322. Dairy Waste Saving and Disposal. W. A. DEAN, JR., Bowman Dairy Co., Chicago, Ill. Milk Plant Monthly, *36*, 5: 42–48, 50. 1947.

Dairy waste disposal is an old problem in which increasing interest is shown today. Reduction of waste in a plant to the minimum is financially beneficial, as well as a means of avoiding possible lawsuits. Plants have been sued for devaluation of property, injury of livestock, discomfort, loss of health and well-being, destruction of fish and wild life, destruction of plant and plankton life, and general nuisance. Dairy wastes may be classified as follows: (a) waste due to inefficient processing equipment, methods, or operation; (b) waste of whey, buttermilk, skim milk or other by-products; (c) waste of spoiled products; and (d) wastes due to rinsings and wash water. Methods of keeping waste to a minimum and the various systems involved in treatment of necessary wastes are described fully. Dairy waste sampling and testing followed by determination of the causes of excessive losses aid in reducing losses. G.M.T.

323. Effect of Stabilizers on Frozen Cream. R. W. BILL, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Dept. of Agr. Jour. Milk and Food Technol., *10*, 3: 149–157. May–June, 1947.

The use of a stabilizer which would make it possible to obtain thawed cream resembling bottled whipping cream was investigated. The stabilizers used were emargol and fruit pectin. These compounds, when added to raw cream, appeared to help conserve the physical stability of the pasteurized

cream during low temperature storage. Emargol tended to decrease the tendency of thawed creams to oil off, without changing the viscosity. Pectin improved the consistency and increased the viscosity of the thawed cream without the tendency to form a cream plug. The differences between the control samples and those which contained a stabilizer were more pronounced when the freezing and storage temperature was – 15° C. than when it was – 29° C.

Emargol and pectin were valuable in stabilizing the cream. However, they were not so important as the size of the fat globules, avoidance of clumping, rate of freezing, and storage temperature. H.H.W.

324. Butterfat and Solids-not-fat. Their Relation to Plant Control and Their Implication in Plant Operations. L. C. THOMSEN, Univ. of Wisconsin, Madison. Milk Indus. Found. Assoc. Bul., *39*, 10: 257–266. March, 1947.

The author suggests that losses be "departmentalized" and not taken merely as over-all plant losses. This is to fix responsibility. If two methods of expressing losses are used, such as per cent of the fat lost and per cent of product lost, then one method may be used as a check against the other and may assist in identifying the point of loss.

In calculating fat losses it is important that the sample in the receiving room be accurate and that the analysis of the original milk samples, as well as the fat content of the various products, yield as near true fat contents as possible. For some products the usual Babcock method is not satisfactory, and a modification must be used. In that part of the milk skimmed, the usual fat loss in the skim milk from 5.0% milk will amount to 1.3% of the fat, while from 3.0% milk it will amount to 2.15%. In working out the losses on the basis of the weight of product, accurate weighing or measuring devices must be provided, containers must have the correct volume, and suitable conversion factors must be used.

The total "paper" losses in ideally operated plants selling only fluid milk and cream will amount to approximately 1.65%, of which slightly less than three-fourths can be assigned to the fluid milk part of the operation and slightly over one-fourth can be charged to the fluid cream operation. The percentage of loss may vary widely from the above if corresponding testing procedures are not used. E.F.G.

325. Plant and Accounting, Their Relation and Contribution to Efficient Management. H. LAGONI, Borden's Dairy Delivery, San Francisco, Calif. Milk Indus. Found. Assoc. Bul., *39*, 10: 281–287. March, 1947.

It is not difficult to determine when costs are too high but it is important that the cause of the high cost be found and the extent to which it is high

actually determined. The various items of cost—such as plant, labor, equipment, materials, supplies, power, steam, refrigeration, waste, and container loss—should be determined on the basis of appropriate units and these costs compared with previously established reasonable standards. This fact-finding operation will furnish the accounting data necessary to a better management job. E.F.G.

326. The Relation of Plant Operations and Accounting to Efficient Management. R. J. RAMSEY, Ramsey Laboratories, Cleveland, Ohio. Milk Indus. Found. Assoc. Bul., *39*, 10: 267–280. March, 1947.

Periodical examination of the records being kept will show some which can be dispensed with and others which are needed. Butterfat accounting is considered to be of first importance, and it should not be difficult to keep these losses below 1% of the fat purchased. Among the procedures recommended for fat checking are the use of a drip sampler for total fat, the use of an automatic sewage sampler, accurate fat standardization of all products, correct weight of product in all containers, and collecting drip, rinsings, and foam for later separation. The per cent of fat in skim milk should be figured at 0.1% as shown with the Mojonnier test.

Bottle trippage is checked by weighing broken glass at critical points and keeping the bottle deposit on wholesale bottles as high as possible. Unless the market is almost ready for paper, it is suggested that substantial savings may be effected with lightweight 48 mm. bottles. Keeping cottage cheese yields up to 16–18 lb. per 100 lb. of skim milk, careful checking of ice box inventory and steam costs, larger use of latent heat equipment, and higher back pressure in the refrigeration plant are important methods of increasing efficiency.

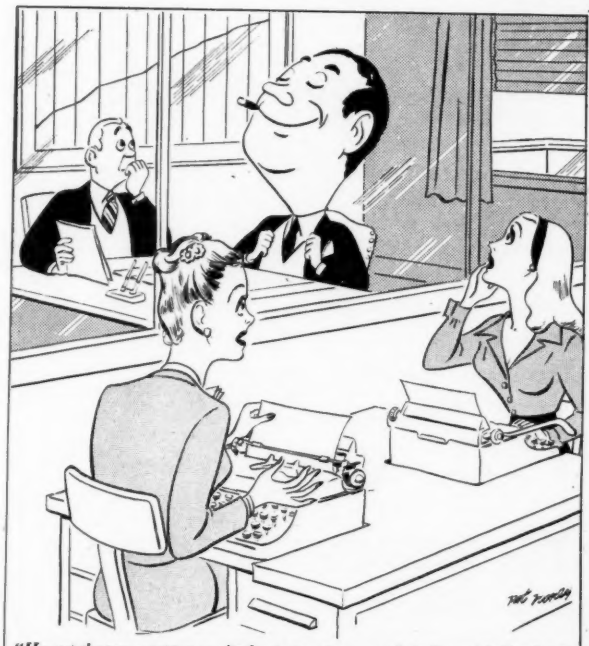
A point to be kept in mind is that it costs approximately five times as much to cool milk one degree as it does to heat it one degree. With S.T.H.T. pasteurization, the use of regeneration may decrease the use of steam to the point that the batch method would require 365% of the S.T.H.T. requirement and in refrigeration the batch method would require 180%, with the S.T.H.T. requirement considered 100%. Industrial water treatment is a source of very substantial savings. E.F.G.

327. How a Safety Program Can Save You Money. E. G. QUESNEL. The Borden Co., N. Y., N. Y. Milk Indus. Found. Assoc. Bul., *39*, 12: 355–360. March, 1947.

The total cost of an accident is on an average four times the direct cost. The all-industry accident rate compiled for 1945 by the National Safety Council shows accidents per million hours worked to be 13.53; for the food industry it was 22.80, while for the dairy products industry it was 26.02.

Accidents in the dairy industry apparently occur almost twice as often as in all industry. The Milk Industry Foundation started a vehicle accident contest Sept. 1, 1939. Of 166 fleets enrolled for the year, only 4 reported no accidents and 36 had less than 5 accidents. The contest was continued each year and the contest ending Sept. 1, 1946, showed 209 fleets enrolled, 19 having no reportable accident and 63 having less than 5 accidents.

A contest for plant workers started in 1941, with 121 companies enrolled, showed 12 with no accident the first year and 52 with less than 5 accidents. In the contest ending Sept. 1, 1946, 21 companies experienced no reportable accident and 90 companies had less than 5 accidents. The contests call attention to safe operating rules and regulations, supply instructions for setting up a safety department in each company, and in many ways draw attention to the importance of safe practice and procedure. E.F.G.



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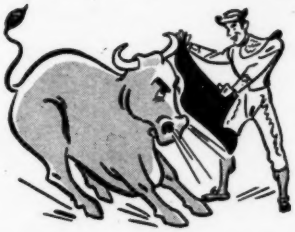

Bulls are color-blind. Scientists have disproved the theory that they "see red." Famous matadors agree that when "El Toro" blows his top at sight of a waving red cape, it's the waving . . . not the red . . . that riles him.

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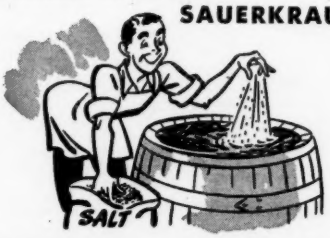

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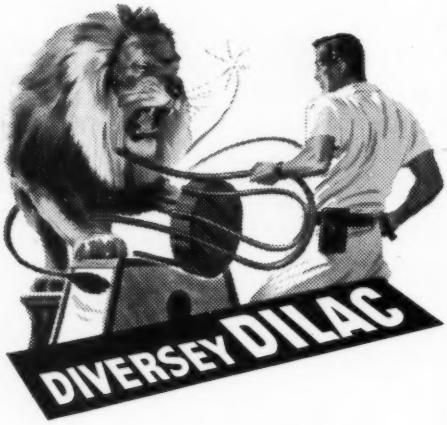

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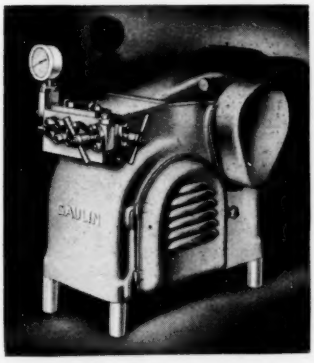

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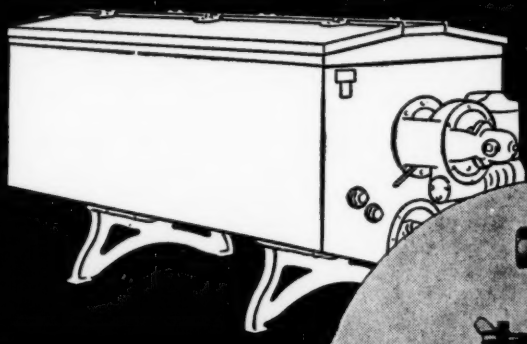

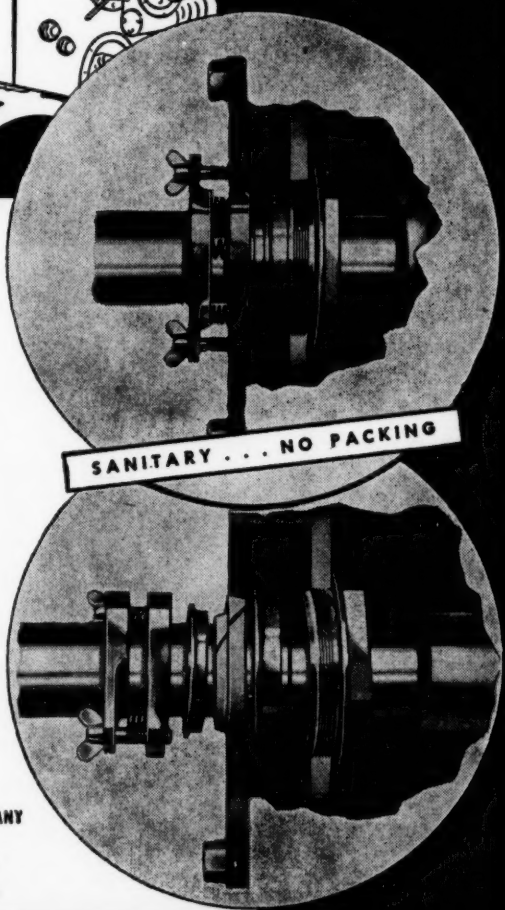

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